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EDITOR'S VIEWPOINT

The measurement of facial swellings has been on a very crude basis, but Björn, Hjelmström, and Lundqvist (p. 295) suggest an accurate photogrammetric method. Application of this technic to studies on the effect of surgical methods and drug therapy should aid in placing such investigations on a more accurate basis. While the method is not simple, nor applicable in general practice, it appears worthy of use where the results of therapy are being evaluated.

Bavetta, Bernick, Geiger, and Bergren (p. 309) placed weanling rats on tryptophane-deficient diets and studied the effects on teeth and bones with and without repletion. The alveolar process showed more severe changes than the femur after a few weeks of tryptophane deficiency. The incisor teeth evidenced disturbances in dentinogenesis but did not show these changes as soon as did the bone. All the changes in bones and teeth were reversible. While these changes were produced by a specific amino acid deficiency, it is doubtful whether they are specific lesions rather than evidence of general protein metabolism upset. Such changes in young human beings as a result of protein deficiency may be masked on repletion but may contribute to malrelationship of the jaws.

Stack (p. 316) was unable to find any correlation between caries activity, measured either by DMF counts or by caries-susceptibility tests, and salivary tryptophane content. These findings on fifty-five individuals are in disagreement with the results obtained by Turner and Crowell. (*J. D. Res.* **26**: 99, 1947.)

Chauncey, Lionetti, Winer and Lisanti (p. 321), to study the enzymes of saliva, determined their presence in whole saliva, cannulated parotid saliva, and oral bacteria. Of ten enzymes present in whole saliva, six were found in parotid secretions, and all but two could be produced by salivary microorganisms. The enzyme system of the oral fluids is complicated because of its multiglandular origin, and contributions from the oral flora, and salivary debris. As the enigma of the salivary enzymes, as well as that of the oral flora, is unraveled, our knowledge of oral health will be greatly enhanced.

Helman and Mitchell (p. 335) were unable to correlate salivary phosphorus levels with calculus deposition or with lactobacillus counts. These findings contradict the claim that salivary phosphatase levels are associated with rate of calculus formation but leave unanswered the possible role of local phosphatase production (in the dentobacterial plaque area) with calculus or lactobacilli. Just as the pH of the saliva has little if any relationship to the local acid production in regions of caries activity, the phosphatase of the whole saliva may not give the answer to phosphatase activity in microregions.

Dreizen, Mosny, Gilley, and Spies (p. 339) found that the absence of any one of six amino acids inhibited acid production by an oral lactobacillus, and the absence of any one of three inhibited acid production by *Staphylococcus albus* although *Streptococcus salivarius* and a saccharomycete had no such requirement, nor did a mixture of the organisms. These, and their other experiments on amino acid requirements, suggest the possibility that differences in nutritional requirements may contribute to variation in acid production by the dentobacterial plaque.

A number of studies of caries incidence in desalivated animals have been reported. Muhler and Shafer (p. 346) found an increase in caries in rats after removal of salivary glands, as had others, but they found the effects greater in females than in males. Further study showed that the desalivation resulted in uterine atrophy and adrenal cortex changes in female rats. These may be due to reduced food intake or to other factors, such as stress. Fluorine was shown to inhibit caries to the same degree in desalivated rats of both sexes. Fluorine storage in the femurs was not influenced by desalivation, but administration of diethylstilbestrol to castrated females increased storage. The interrelationship of caries desalivation and hormonal activity appears confusing, and more definitive experimentation is indicated.

Perhaps one is dealing with stressed animals in which the adaptation obscures the results of removal of glands.

Manhold and Rosenberg (p. 357) have attempted to correlate DMF with personality variables. While some relationship was found between certain personality traits and the number of cavities present in 266 Naval Air Cadets, the findings were only weakly significant and should be considered as suggestive, not definitive.

Injection of propylthiouracil produces an effect similar to that of thyroidectomy in experimental animals. In rats, Paynter (p. 364) demonstrated quantitative but not qualitative effects on the teeth and jawbone by use of this drug. Although some authors had described effects on the quality of dentin and enamel following thyroidectomy, this was not duplicated in the nonsurgical procedure of reducing thyroid activity. It is suggested that the surgerized animals were accidentally parathyroidectomized, or that surgery interfered with parathyroid activity. The only other obvious explanation would be based on a dual endocrine activity of the thyroid with thiouracil suppressing the hormone controlling quality but not that effecting quality. However, the hormonal activities of the thyroid have not been so differentiated.

English, Schlack, and Ellinger (p. 377) directed ionizing radiation at the heads of rats and studied the effects, over a period of time, on the incisors. The severe effect resulted in a radiographically visible break in the continuity of the continuously erupting incisors. The older segment was delayed in eruption, and the "new" segment was disturbed in its reorganization by the irradiation. Grossly, the separation of the segments led to appearance suggesting extra lower incisors and short or absent upper incisors. The radiation was not so localized that it could not have effected the pituitary. The disturbances in eruption should not only be considered from the viewpoint of the local growth disturbance but also from the broader viewpoint of hormonal (pituitary) dysfunction.

Kalnins (p. 389) observed pulpal changes after irradiation. The pulps, in some instances, were protected by platinum shields so that the indirect effects on the pulp could be studied. Changes observed in the protected radicular pulps included telangiectasis, hemorrhage, edema, "collagenous coats" about vessels, fibrous metaplasia, and endothelial swelling. The author's findings suggest that mature odontoblasts are among the most radioresistant cells.

Phillips, Schnell, and Phillips (p. 400) report the early phases of an investigation into the electric conductivity of cement. It has long been accepted that electric currents flow about metallic restorations, and dental cements have been regarded, generally, as insulators of the dental pulp. Like many such procedures, the application has been on empiric grounds or strictly conjectural. This study presents an in vitro method for study of the effects of composition, aging, preparation, and other factors on electric conductivity of dental cements.

There has been much discussion of investigations in the dental restorative materials of the plastic type. Crawford and Larson (p. 414) present their findings on amalgams and acrylic resins. The authors approach this problem in an effort to find acceptable clinical materials. It is interesting that, after a thorough study of amalgams and plastics, the hope is expressed that a new material which will be entirely satisfactory will be found.

Margolis and Prakash (p. 425) describe an instrument for recording oral muscle forces. Numerous instruments have been devised for study of such forces, but none has proved entirely satisfactory. This new refinement may offer advantages to make it suitable in hands other than those of its designers.

Meredith and Hixon (p. 435) studied the cusp of Carabelli by devising a scale of classification and applying it to casts from 100 children 9 years of age. Some protuberance was found in 84 per cent of the 200 maxillary first molars. Such studies are often overlooked as unnecessary, or as unimportant. There are a great many statistical studies of this type which need to be carried out. They should be encouraged as contributions toward the completeness of knowledge of oral structures and diseases.

H. B. G. R.

A PHOTOGRAMMETRIC METHOD OF MEASURING THE VOLUME OF FACIAL SWELLINGS

HILDING BJÖRN, L.D.S., M.D., CLAES LUNDQVIST, L.D.S., D.D.S., M.D., AND PETTER HJELMSTRÖM, C.E.

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IT IS desirable from various points of view to be able to measure quantitatively the volume of facial swellings in order to follow their development and to determine objectively their magnitude and duration. Such a measuring method would be of value when judging how the various etiological and pathogenetic factors influence postoperative edema, infectious swellings, edema resulting from local anesthetics, and so forth. A method of this kind, applicable to the jaw area, has, so far as we know, not yet been described.

The method of recording volume changes of the body seems on the whole to be little used. An example, however, of a technic which has been employed, especially in physiology, is the plethysmographic method described by Lewis and Grant (1925) and Prinzmetal and Wilson (1935). For measuring rapid volume changes, a so-called impedance plethysmograph can be used. This method is based on the fact that the impedance of an extremity, for example, is under certain measuring conditions a function of its volume (Nyboer, Bagno, and Nims, 1943).

The authors did not consider this or any similar methods appropriate for their case, since such methods are concentrated on volume variations of short duration, and since practical difficulties are encountered when applying them to the jaw.

Photogrammetric methods were considered more suitable when dealing with facial volume mensuration, mainly because the measuring can be effected without any noteworthy influence on the object. In medicine and dentistry, photogrammetry has been made use of by Laemann (1950), Berghagen (1951), Adams-Ray and Hjelmström (1951), and others. Volume measurements of the direct type, as used in the experiments reported here, do not seem to have been previously applied to biologic objects, but have been used for quite different objects, for example, coal heaps, and so forth (Hallert, 1949).

The purpose of the investigation was to make a practical test of the photogrammetric measuring method for the determination of facial swellings, to elaborate this method, to demonstrate as far as possible its accuracy, and to apply the results to cases of postoperative swelling.

This work has been supported by grants for dental research from the State Dental School, Malmö, Sweden.

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GENERAL INTRODUCTION

Volume Measurements.—The measuring of volume involves the determination of how many times the volume unit is contained in the volume to be measured. The volume of a rigid body is enclosed by its surface, which is also rigid, and consequently has a definite shape. The knowledge of the shape and size of the surface makes it possible to determine the volume enclosed. If the rigid body has a simple geometric shape (cube, sphere, and so forth), simple geometric formulas can be employed for the determination of its volume, but if its shape is irregular, a calculation by this method becomes impossible. In such difficult cases the procedure involves a division of the body into parts of simplified volumes (volume elements) and their addition. Greater accuracy often requires very small volume elements, and the addition then becomes integration (the addition of an infinite number of infinitely small quantities).

Stereophotogrammetry.—Photogrammetry deals with the problem of determining metric properties in objects with the aid of photographs. Here, metric properties refer to the shape, size, and position of the object, which are found by the aid of angles, lengths, surfaces, volumes, and so forth. Stereophotogrammetry is an important part of photogrammetry. The use of stereoscopic vision has led to the construction of more or less automatic instruments (autographs, planigraphs, and so forth), with the aid of which the previously mentioned photogrammetric tasks can be solved. Photogrammetry is mostly used in the field of cartography, but it can also be used to advantage in quite different spheres. Its method of operation is roughly as follows: The object to be measured is photographed from two different points to obtain different perspectives of the same object. The pictures are placed in the stereoinstrument, and are by orientation brought into the same conditions as when the photographs were taken. They are observed through the twin eyepiece of the instrument, each eye seeing the corresponding image only. Because of the difference in perspective, the observer gets a three-dimensional impression of the object photographed; that is, an impression similar to that which he would have received had he looked at the object directly, with his eyes placed at the points from which the two photographs were taken. Generally, those two impressions are not identical owing to the properties of the optical system, but the difference is irrelevant from a measuring point of view. The observer is said to be viewing a stereomodel of the object.

The measurements are taken by means of the so-called "floating mark." This is based on the following fact: Every detail of the object has distinct positions on the two photographs. Consequently, a definite connection is established between the positions of the detail on the photographs and its position on the object. In the field of vision of each eye there is a little mark, which is seen simultaneously with the image, and whose position can be adjusted at will. If the marks are placed on corresponding details in both pictures, the observer is unable to decide whether the marks themselves are details of the image or not. His stereoscopic impression is a single mark (the floating mark), whose position in space he perceives. The mark is adjusted

to the surface of the object in question. The stereoinstrument is so contrived that the connection between two identical details in the two photographs and the corresponding detail in the object determines the movement of a certain device in the instrument in relation to the movements of the two marks on the image. This instrumental detail thus carries out the actual spatial movements of the floating mark, which the observer perceives stereoscopically. The position of the device in question can be mechanically measured, and its movements automatically recorded. By adjusting the floating mark to the various details of the object, the position of these can be measured.

A common method to find out the shape of the surface of the object is to draw contours (curves of intersection between surfaces and planes). A succession of curves of intersection between the surface of the object and a series of parallel planes with equal distance are drawn on the same paper. A family of curves is obtained, the shape and density of which give a clear picture of the shape of the surface. This technic is usually adopted in maps to illustrate the varying heights of the terrain. It can be said that the volume contained by the surface of the object is divided up into layers by the intersecting planes. The technic is thus also suitable for volume recording of objects with irregular surfaces. If the surface enclosed by each contour is measured with a planimeter, and the thickness (equidistance) of the layer is known, an approximate value of the volume of each layer can be obtained. These volumes are then added and an approximate value of the total volume is obtained. The accuracy of this procedure naturally depends on the accuracy with which the volume of the layer is calculated.

Application of Stereophotogrammetry to Facial Swellings.—The first problem to be solved concerned what kind of closed surface would be most suitable for the volume measurings. A closed surface of the smallest possible extent, but nevertheless containing the whole swelling of the face, was wanted.

TECHNIC

Apparatus.—

1) *For photographing:* Wild's Stereocamera, No. 165, base = 40 cm., focal distance = 9 cm. Extra lenses were used to focus the camera at a working distance of 80 cm. Negative material: plates 6.5 by 9 cm., Gevaert Superchrome 32°. Illumination: two synchronized electronic flashlights.

2) *For measuring:* Wild's Autographs A5 and A6 together with a planimeter (Coradi).

Procedures.—

Photographing: An impression of the patient's upper and lower teeth was taken in self-curing acrylic resin on a U-shaped brass plate. The plate was left to harden and was then attached to a stand, which in its turn was fastened to a steady table, which also supported the stereocamera (Fig. 1). During the exposure the plate could be fixed with high accuracy in the same position

on the stand, and the patient had to bite into the impression. The camera was placed, as is seen in Fig. 1, on one of the two disks at the ends of the table, so constructed that the same position could be reproduced with high accuracy. To support the stereowork, three marks were set up, one on the stand of the bite plate and two on a special pillar fixed to the table. These points formed a triangle which, as seen from the camera, roughly framed the part of the patient's face which was to be examined.

Fig. 1.—Stereophotographing of the face. The patient is fixed by biting together on the bite-impression attached to the table (definitive device).

Measuring procedure: The negative pictures were measured in a Wild Autograph (most of them in the A6). Since the stereoinstrument does not allow a focal distance of 9 cm., the measuring was carried out with different scales for height and plane. The equidistance corresponded to about 2 mm. in reality. During the experiment, the position of the camera was always the same in relation to the head. The consequence of this arrangement was that

surfaces with fixed positions in the object also received fixed positions in the stereoinstrument. The volume to be measured could thus be enclosed within certain surfaces with fixed positions in the stereoinstrument and one surface containing the swelling deformation (Fig. 2). Actually, this was performed by drawing straight lines in one plate holder, defining planes through the center of projection (that is, the corresponding camera lens). Another plane

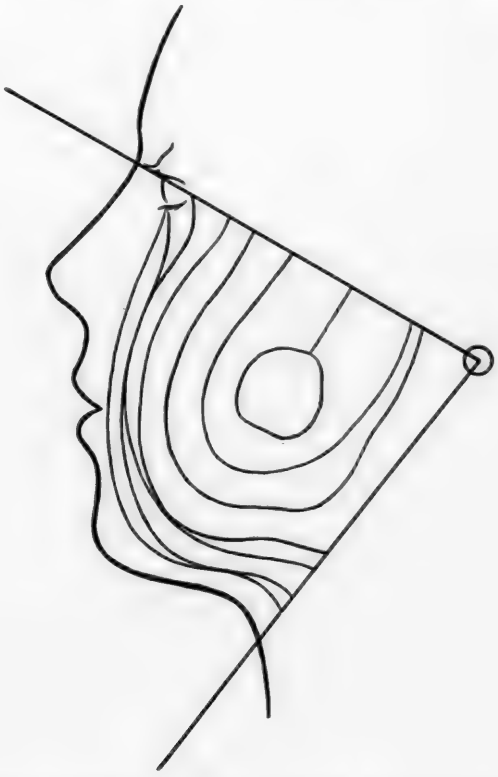

Fig. 2.—Sketch showing delimitation of a section within the face for measuring the volume.

parallel to the plates could be held in a fixed position by adjusting the elevation scale of the instrument. These planes, together with the partially deformed facial surface, constituted the closed surface to be considered. The volume enclosed could be measured by the integrating procedure described previously, since it was possible to follow its surface with the floating mark. The planimeter was connected to the instrument and, therefore, the integration was made automatically.

CONTROL MEASUREMENTS

Determination of Accuracy.—This part of the work involved a number of special investigations. The properties and orientation of the camera were

studied, as well as the working method of the autograph and the planimeter. To discuss these investigations in extenso here would demand too much space, but in order to give the reader an idea of the possibilities of the method, certain results are furnished. The volume variations caused by deficiencies in the stereowork were controlled by repeating the plotting of the same pair of pictures ten times. The delimited volume had an average size of 58.4 cubic centimeters, with the divergences of +0.1, −1.2, +0.6, −0.6, −0.2, +0.9, −0.2, −0.5, +1.5, and −0.4 cubic centimeters, from which $\sigma = 0.8$ cubic centimeter was calculated. The influence from deficiencies in the orientation of the patient was studied by placing him in the apparatus ten times, taking photographs each time. At the plotting, this series of stereopictures provided the following series of divergences from the average of 69.7 cubic centimeters: +0.3, −2.0, +0.1, +1.1, +2.4, +2.3, −2.9, −0.6, −0.9, and +0.1 cubic centimeters, $\sigma = 1.7$ cubic centimeters.

Fig. 3.—Photogrammetric measurement of a known volume of putty marking "swelling" on a bronze head.

Control Against Gross Errors.—As a means of control against gross errors in the measuring method, the following tests were performed. A bronze head (Fig. 3) was photographed in the same way as that of the patient, with a lump of putty applied, modeled to resemble a postoperative swelling, and also without this application. The volume of the putty was determined by weighing it, the specific weight of the material being already known. This experiment was

repeated with various lumps of putty, whereupon when the variations of volumes, measured by the photogrammetric method, were less than the smallest standard deviation given in Table I, the absence of gross errors could be ascertained.

TABLE I

COMPARISON BETWEEN KNOWN AND PHOTOGRAMMETRICALLY MEASURED VOLUME: TEST WITH PUTTY ON BRONZE HEAD

OBJECTS MEASURED	KNOWN VOLUMES ($CM.^3$)	PHOTOGRAMMETRICALLY MEASURED VOLUMES ($CM.^3$)
"Swelling A"	15.45	15.15
"Swelling B"	20.07	19.54

IMPROVEMENTS

The arrangement of the experiment, as described previously, was the result of a number of modifications undertaken in the course of the work in order to improve the accuracy. The most important of these changes are reported in the following description of the first experiments. The patient was fixed in a cephalostat (Fig. 4) by plugs pushed into the auditory canals, by supports for the nose and the neck, and by an incisal pin. The positions of the supporting aids could be read off on scales in order to make a resetting in the same position possible. The camera was fixed on a tripod, the position of which was determined by metal marks on the floor. The determination of accuracy showed that this method of arranging a fixed relation between patient and camera was inferior to the earlier-described procedure. The standard deviation in the volume measuring amounted to $\sigma = 3.2$ cubic centimeters instead of the previously mentioned figure of 1.7 cubic centimeters. Here, also, the stereowork was more uncertain: $\sigma = 1.8$ cubic centimeters instead of the previously mentioned figure of 0.8 cubic centimeter. The latter difference was considered, among other things, to be due to faulty fixing of the patient in the cephalostat and to the fact that the working distance used was about 10 cm. too far, which gave rise to indistinct photographs and less geometric accuracy in the instrument. Inaccuracy in the position of the patient in the cephalostat was proved by direct measurements in the stereocomparator. These measurements showed that there was an obvious risk of a volume error of about 4 cubic centimeters. The actual stereowork was varied in many respects in order to find a suitable and convenient method of procedure.

DISCUSSION OF METHOD

The degree of accuracy of the method was determined, and was roughly doubled. The main reason for the increase of accuracy was the substitution of the fixing of the patient by the aid of a bite-impression for the fixing in a cephalostat, a result which ought to be of interest in many other biometric connections. The time employed to measure one volume averaged thirty minutes.

In order to estimate the applicability of the method to the study of swellings, a closer examination had to be made of the earlier-mentioned condition that the variations in the delimited volume must be representative. The

Fig. 4.—Device for identical stereophotographing of the face. Patient fixed in the cephalostat (preliminary arrangement). Lighting: electronic flashlights.

method used gives, as a result, that volume which is enclosed between two borders of the facial surface. It is easily understood that a surface swelling extending beyond the delimited area, as well as an inner swelling, cannot be recorded in this manner. When judging the results, these circumstances must be taken into account. In the case under consideration, swellings of a very local type were recorded, whose spread beyond the delimited area probably

was without great significance. It is, however, much more difficult to express an opinion concerning an inner swelling.

It is also possible that the change of shape in the delimited area can be due to something other than swelling, for example, various stages of contraction of the muscles.

A fact which must be taken into account here is the variation in the position of the lower jaw. A tight biting together gives rise to contraction and consequently a change of volume in the masseter muscle and in the cheeks. The physiologic rest position of the mandible involves a certain distance between the rows of teeth, which occasions a change in the configuration of the face compared with the previous situation. The part played by the opening movement is still more accentuated if the postoperative swelling and pain prevent the patient from clenching his teeth. In excentric positions of the lower jaw, the contour of the cheeks can, of course, be greatly changed.

When the patient was fixed in the cephalostat, the position of the lower jaw was not under full control, which, however, was the case at the improved fixation with an acrylic impression, around which both jaws had to bite together. This was decidedly an advantage. A fixation with the patient's lips apart also effectively prevented the cheek from being blown out, which could be the case when he had to keep the mouth shut. It was, of course, desirable that the muscles of facial expression should be relaxed as far as possible when the photographs were taken.

On the basis of the experiments performed, we believe that the measuring method described can yield good results in clinical application if necessary precautions are taken with regard to the position and behavior of the patient. A few applications of the method to cases of oral surgery are described.

RESULTS OF VOLUME MEASUREMENTS OF POSTOPERATIVE FACIAL SWELLINGS

The purpose of the application was to determine the size of postoperative edema and to express the course of the swelling objectively.

A fairly uniform material was obtained by studying postoperative edema after surgical removal of totally or partially impacted mandibular third molars. The photographs were taken immediately before and after the operation, and then at intervals of twenty-four hours, until the swelling, to the observer, seemed to have subsided.

The technic of operation was as follows: (1) Local anesthesia with 2 to 4 c.c. of 2 per cent Xylocaine-epinephrine. (2) Exposure of the unerupted tooth and its covering jawbone by raising a mucoperiosteal flap. (3) Sufficient removal of bone substance to permit the elevation of the tooth, possibly after division. (4) Application of 40 to 50 mg. of sulfathiazole microcrystalline substance in the alveolus. (5) No postoperative treatment was made, but analgesics were prescribed.

After the surgical removal of the unerupted third molars, a measurable swelling of the cheek regularly ensued (with the exception of Case 15).

Eleven patients were examined by the cephalostatic method. Because of various difficulties, which had nothing to do with the technic as such, only five cases could be followed for sufficient time to give an idea of the course of the curve of swelling. The other cases, however, formed a valuable basis for the development of the method. One of the five cases was not very successful from a technical point of view, probably because of too great a divergence when placing the patient's head at the various occasions of investigation. The results are given in Table II.

TABLE II

RESULTS FROM STEREOPHOTOGRAMMETRIC VOLUME MEASUREMENTS OF FACIAL SWELLINGS IN ELEVEN SERIES AFTER SURGICAL REMOVAL OF LOWER WISDOM TEETH; PRELIMINARY TESTS (VOLUME, CUBIC CENTIMETERS)

	OPERATING DAY		DAYS AFTER OPERATION						
PATIENT	PREOP.	P.O.	1	2	3	4	5	6	7
1	0	− 6		2	5	2	4	2	7
2	0	13	10	14	32	27	7	11	
3	0	5	6	11	4	2	2		
4 right	0	−10	−13	−13					
4 left	0	1	2	11					
5	0	7	9	16	14	13	2		
6	0	− 4	− 2						
7	0		12	3					
8 right	0		5	8					
8 left	0		10	7					
9	0	18	16	24	21	15	−3		

Thirteen test series were carried out on twelve patients with the improved fixation technic. One case was excluded because of faulty biting, with uncontrollable orientation variations. One of the remaining 103 pairs of stereopictures was excluded because of poor photographing.

The results of the volume measurements are collected in Table III and shown in Figs. 5 and 6. The volume values are given in relation to the preoperative condition.

TABLE III

RESULTS FROM STEREOPHOTOGRAMMETRIC VOLUME MEASUREMENTS OF FACIAL SWELLINGS IN TWELVE SERIES AFTER SURGICAL REMOVAL OF LOWER WISDOM TEETH; PRINCIPAL TEST (VOLUME, CUBIC CENTIMETERS)

	OPERATING DAY		DAYS AFTER OPERATION											
PATIENT	PREOP.	P.O.	1	2	3	4	5	6	7	8	9	10	11	12	13
10	0	4	24	25			6	6							−6
11	0	5	27	24	16	15	9	4	2						
12	0	5	14	16	13	12	10		7						
13	0	8	6	10			3								
14 right	0	0	8	8	3	4	5		−2						
14 left	0	3	19	17	14	8	9	8	2						
15	0	−2	2	3	0	−2		−1	−4	−4	−5				
16	0	2	30	29	24	12	9	0	3	−1	3				
17	0	11	27	21	14	13	8	8	9						
18	0	−2	17	14	10		4	0							
19	0	4	16	10	7		3	4	1						
20	0	3	32	26	25	15	16	9	9	8	4				
Mean value	0	3.3	18.5	16.9	12.6	9.6	7.5	4.2	2.7						

As a rule, the swelling reached its maximum after one to two days, which is marked in the diagrams by a peak or a level. The edema then subsided fairly rapidly.

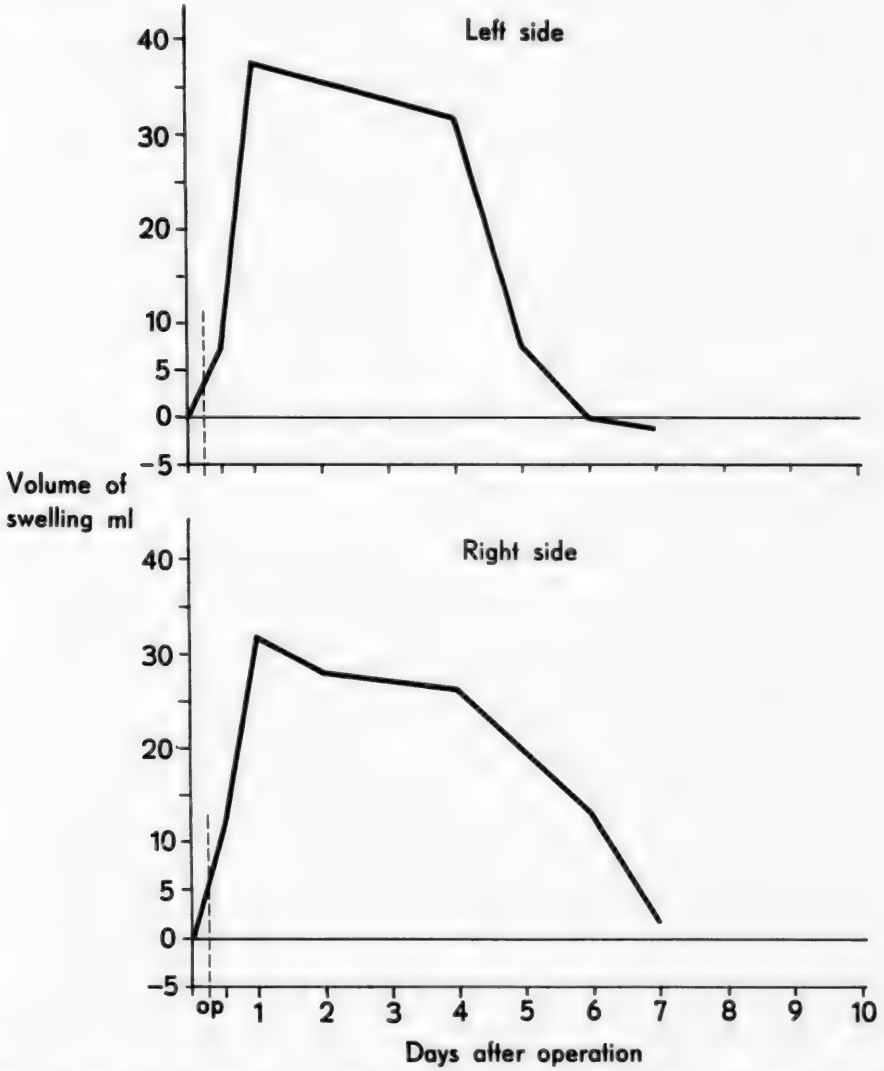

Fig. 5.—Course of swelling in a patient in whom both unerupted mandibular third molars have been surgically removed.

The degree of swelling varied from practically no measurable swelling (Case 15) to a maximum swelling of 32 cubic centimeters (Case 20). In the cases where a swelling arose, its maximal volume reached, on an average, 20 cubic centimeters (18.5 cubic centimeters for all the series).

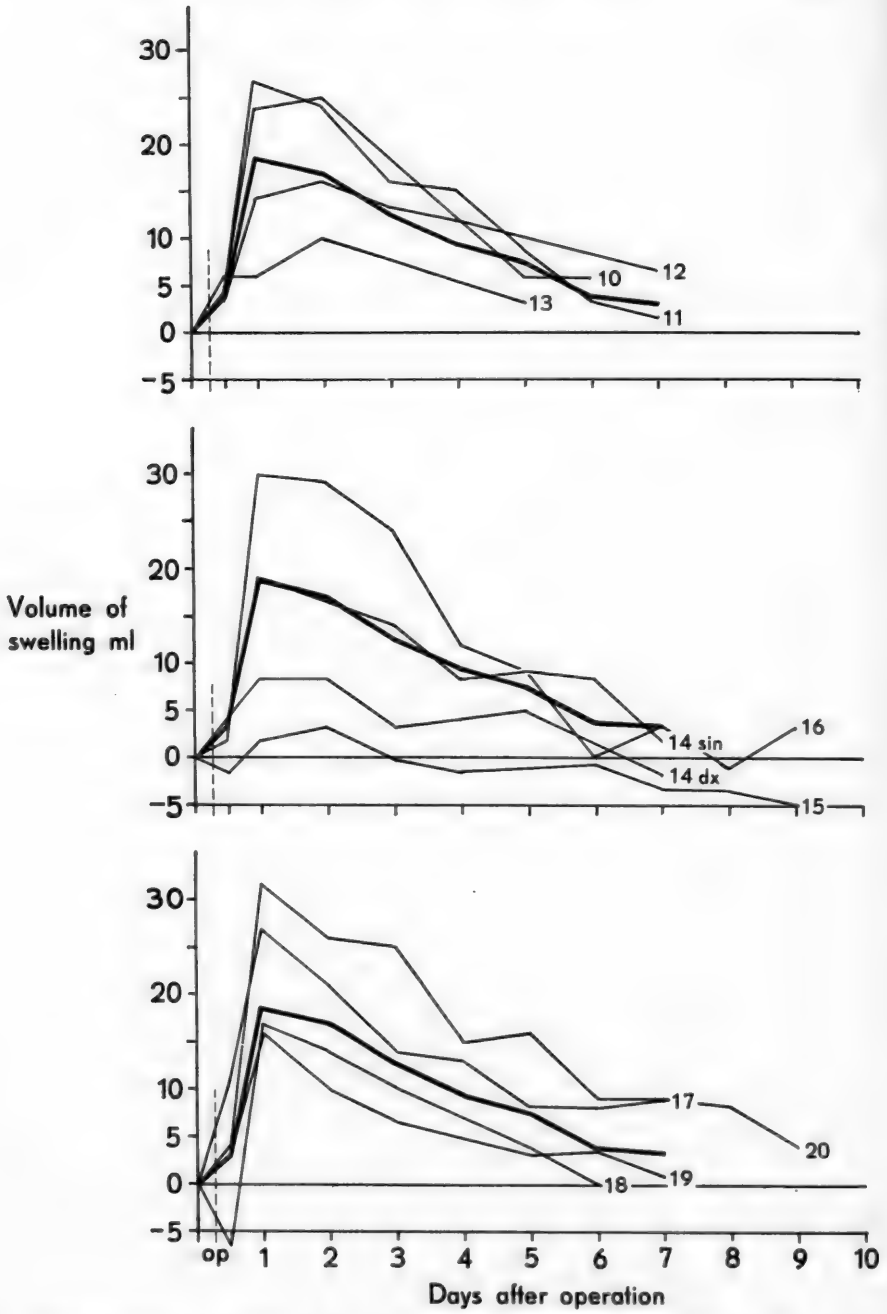

Fig. 6.—Course of swelling in the face after surgical removal of mandibular third molars. Four photoseries are given in each diagram. The mean value is calculated for all twelve series (Table III).

The duration of the swelling was, of course, of great interest. The curve of duration assumed an asymptotic course at the recession of the swelling. Because of this it was, of course, impossible to decide with due accuracy the point of time when the swelling had ceased. It was, therefore, most suitable to report the duration as the time elapsing before the swelling had gone down to a certain value or to a certain fraction of the maximum value. If the latter method is chosen, and one-fourth of the maximal swelling is set as a limit, the duration of the swelling in this experimental series was five to nine days, the great majority of cases showing a duration of five to six days.

DISCUSSION

A comparison of the experimental values of the preliminary and the finally adopted methods presents a more reliable evenness in the volume results of the latter. This is in full agreement with the reduction of method errors, which has already been discussed.

When recording volume differences on the face, lower values than those previous to the operation can be obtained after the subsidence of the edema. This might be due to a preoperative edema, originating from the pericoronitis that indicates the operation, but could possibly also be ascribed to the patient's losing weight during the period of examination. A loss of weight is, indeed, very often observed in connection with difficulties in taking food or as a result of pathologic processes and operations in the mouth. The possibility of a general retention of fluid, or the reverse, should also be taken into account when adapting the method to certain clinical material.

The course of the swelling can vary under the influence of several factors, which were perceived only in some of the cases. A delay may occur by reason of secondary infection of the wound, which should be revealed by checking the temperature, leukocyte count, and so forth. The fundamental cause of this delay is probably operative trauma, which can be aggravated by the effects of local anesthetics or the presence of infection or postoperative hemorrhage in the tissue.

The aim of the investigation described in this paper was not to establish the importance of the factors in relation to one another and to the casual connection. However, a method has been worked out, which ought to be a rich soil for further studies.

SUMMARY

A stereophotogrammetric method was used for a quantitative determination of volume variations in a section of the face. This method involved volume measuring by the aid of stereophotographs in accordance with principles that are well known in aerial cartography. After the appropriate contouring, the volume was read off on a planimeter fastened to a stereoinstrument. The persons to be examined were fixed by means of a bite-impression, which made it possible to place them in the same position in relation to the camera at successive experiments. The standard deviation of a volume measurement was estimated to 1.7 cubic centimeters.

We are indebted to Professor Bertil Hallert, Chief of the Department of Photogrammetry, Royal Institute of Technology, Stockholm, for valuable advice in connection with this work.

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THE EFFECT OF TRYPTOPHANE DEFICIENCY ON THE JAWS OF RATS

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INTRODUCTION

THE problem of whether nutritional deficiencies in early childhood may lead to irreversible damage is of the utmost importance. This has been emphasized by Hartroft and Best,[1] who demonstrated that feeding a choline-free diet for a short period to infant rats resulted in severe vascular changes in later life and subsequent development of hypertension. Our investigation was designed to determine whether the formation of bones and dental tissues may suffer irreversible damages by temporary inhibition of growth through a deficient diet.

The general consequences of protein and amino acid deficiencies are well known. However, the relationship of amino acid intake to the development and maintenance of the teeth and their periodontium has not been fully investigated. A survey of the literature reveals a paucity of studies which have been conducted on either protein or amino acid deficiencies as related to the dental structures. Hunter[2] reported the effects of methionine, lysine, and total protein deficiency on rate of eruption and incisor formation in the rat. He found no decrease in the rates of eruption of the rat incisors in any of the three types of deficiencies induced, nor was he able to demonstrate, histologically, any pathosis in the teeth, although other tissues, such as the liver and cardiac muscle, showed pathologic changes. The conclusion was reached that the rat incisor enjoys a priority over the rest of the body tissues. However, fully mature animals were used in this study, and the conditions during growth are known to be more exacting. Adamstone and Spector[3] reported significant changes in the liver, testicles, and visceral and cardiac muscles as a result of tryptophane deficiency in the rat. However, the bone and teeth were not investigated. They used the forced feeding technic to accelerate and aggravate the symptoms. However, one wonders whether this method introduces more difficulties than it solves. Schwartz, Scott, and Ferguson[4] studied the effects of phenylalanine deficiency in rats, and reported marked changes in bone, which were reversed in the repleted animals. Following protein deprivation, Chawla and Glickman[5] found degeneration of the connective tissue of the gingiva and periodontal membrane, osteoporosis of al-

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veolar bone, and retardation of cementum. The femurs of the animals were also osteoporotic, and presented a picture of marked inhibition of endochondral bone formation.

MATERIALS AND METHODS

Forty 21-day-old female weanling rats of the University of Southern California strain were used in this study. These were selected from ten litters in order to ensure proper distribution among the experimental groups. The experimental groups were as follows: Group I, tryptophane-deficient diet for three weeks and then repleted for four weeks; Group II, tryptophane-deficient diet for seven weeks; Group III, control diet for seven weeks. All three experimental groups were maintained for one week after weaning on standard Purina ration. At the end of this time (twenty-eight days), the average weight for the individual groups was sixty-seven grams.

An acid hydrolysate of casein was used as the source of nitrogen in the present investigation inasmuch as such a preparation has been demonstrated to cause almost complete removal of tryptophane. The composition of the diet is given in Table I.

TABLE I

COMPOSITION PER KILOGRAM OF DIET

1. Casein hydrolysate	150 Gm.
2. Mazola oil	90 Gm.
3. Wheat-germ oil	40 Gm.
4. U.S.P. salt mixture	40 Gm.
5. Starch	674 Gm.
6. Trace elements*	
7. Vitamin mixture†	

*$MnSo_4.2H_2O$, 100 mg.; $CuSO_4 \cdot 5H_2O$, 40 mg.; $ZnSO_4$, 40 mg.; $CoCl_2$, 2.0 mg.

†Choline, 5.0 Gm.; inositol, 1.0 Gm.; ascorbic acid, 100 mg.; thiamine HCl, 12.5 mg.; riboflavin, 25 mg.; niacin, 500 mg.; pyridoxine HCl, 12.5 mg.; calcium pantothenate, 6 mg.; folic acid, 6 mg.; biotin, 2.5 mg.; menadione, 4.0 mg.; B_{12}, 20 gamma.

The control animals were provided with a diet in which the casein hydrolysate was supplemented with tryptophane to the 0.75 per cent level. At the end of the three- and seven-week periods, equal numbers of animals were sacrificed from all three groups. At the time of sacrifice, the heads and femurs were removed and fixed immediately in Bouin's fixative for at least four days. The femur was included as an indicator system because of the well-known changes that it undergoes during disturbances in calcification. Decalcification was subsequently carried out with 10 per cent nitric acid in formalin, and the specimens were prepared for nitrocellulose embedding by the routine manner. The tissues were then sectioned 20 to 30 micra thick and stained by several different methods. These included Mallory's connective tissue stain, hematoxylin and triosin, and Pearson's silver gelatin impregnation method.

OBSERVATIONS

Gross Findings.—At the end of the three-week depletion period, the animals in both Groups I and II averaged forty-eight grams in weight, while the control animals averaged 120 grams. At the end of seven weeks, the control

animals averaged 180 grams, while those in Group II kept on losing weight so that they averaged only forty grams. However, although the repletion animals did not attain the weight of the control group by the seventh week, they did show a greater rate of increase. During the four-week repletion period, the control animals averaged nineteen grams. The experimental animals, in addition, showed alopecia and cataract formation, which were very marked by the end of the seven-week experimental period.

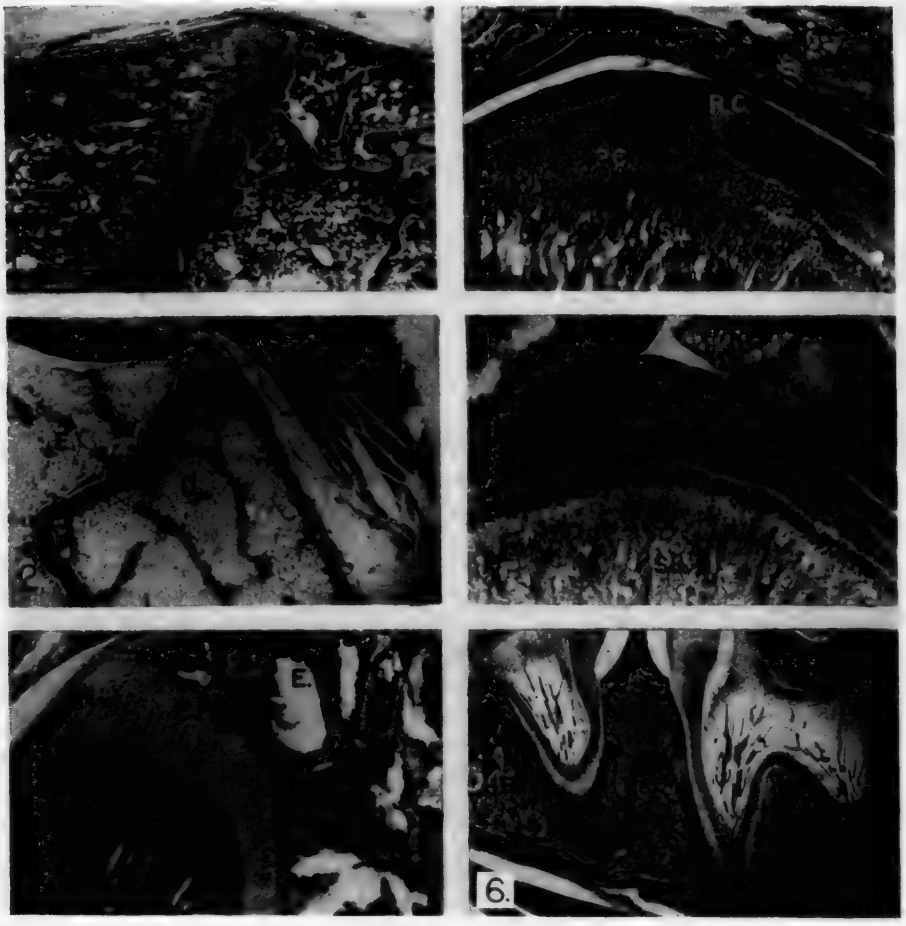
Microscopic Findings.—

Long bones: The femur in the control animals was found to exhibit well-developed trabeculations in both the epiphyseal and diaphyseal areas, with marrow spaces filled with hematopoietic tissue (Fig. 1). Between these two regions the epiphyseal plate clearly shows the characteristic four-zone division. From the epiphysis to the diaphysis these zones are: (1) zone of resting cartilage, (2) zone of proliferating cartilage, (3) zone of hypertrophic cells, and (4) zone of calcifying cartilage. Degenerative changes are seen in these regions at as early as three weeks. The trabeculae in both the epiphysis and diaphysis are reduced in width and number, and show decreased osteoblastic activity. The epiphyseal plate also shows a definite narrowing, with atrophy in the zone of proliferation. There is beginning replacement of myeloid tissue by fatty tissue. However, the reversibility of these lesions is evidenced in Fig. 3, which represents a section through the head of the femur of a four-week repleted animal, showing return of trabeculae into both the epiphysis and diaphysis. The increased osteogenic activity is indicated by the thick spicules and layers of active osteoblasts. The active resumption of chondrogenesis is emphasized by the thickened epiphyseal plate, particularly at the zone of proliferation. There is also a replacement of the adipose tissue by the myeloid elements so that hematopoiesis is again active.

There is an aggravation of the deficiency symptoms in the long bones as seen at seven weeks (Fig. 2). There has been a disappearance of the trabeculae in both the epiphysis and diaphysis, so that only one or two spicules are visible in the field. There is an extreme thinning of the epiphyseal plate in which the zone of proliferation and hypertrophy have atrophied, so that these consist of only two to three layers of cells. Instead of containing elongated strands of cartilage projecting into the diaphysis, the diaphyseal border of the epiphyseal plate is lined with a thin layer of bone.

Mandibular condyle: The silver impregnation of a section of the mandibular condyle in the control group demonstrates the existence of two distinct zones. These consist of a superficial zone of immature cartilage (*R.C.*) cells and chondroblasts and of an inner layer, which is composed of proliferating and maturing cells (*P.C.*). These two regions are separated by a distinct line (Fig. 4).

In contrast to the control animals, the condyle of the experimental animals is smaller in size (Fig. 5). The superficial layer (*R.C.*) appears slightly narrowed, while the inner layer (*P.C.*) has atrophied as a result of the failure of the proliferating cells to mature. The subchondral area, however, appears



KEY TO ABBREVIATIONS:

A.B.—Alveolar bone
D.—Diaphysis
De.—Dentin
E.—Epiphysis
En.—Enamel
E.P.—Epiphyseal plate
I.R.—Interradicular area
I.S.—Interseptal area
P.—Periodontal membrane
P.C.—Proliferating cartilage
Pu.—Pulp
R.C.—Resting cartilage
S.—Spongiosa
Su.—Subchondral area

Fig. 1.—Control animal. Head of femur showing epiphysis, epiphyseal plate, and part of diaphysis. (Hematoxylin and triosin. ×250; reduced ⅗.)

Fig. 2.—Experimental animal on deficient diet for seven weeks. Head of femur. Note decrease in width of epiphyseal plate and disappearance of trabeculae in epiphysis and diaphysis. (Mallory's connective tissue stain. ×250; reduced ⅗.)

Fig. 3.—Repleted animal. Head of femur. Note reappearance of trabeculae in epiphysis and diaphysis, and accentuated widening of epiphyseal plate. (Hematoxylin and triosin. ×300; reduced ⅗.)

Fig. 4.—Control animal. Head of condyle. (Pearson's silver impregnation method. ×250; reduced ⅗.)

Fig. 5.—Experimental animal on deficient diet for three weeks. Note thinning of proliferating cartilage layer. (Pearson's silver impregnation method. ×250; reduced ⅗.)

Fig. 6.—Control animal. Interseptal and interradicular areas between two molars. Note well-formed bony trabeculae in spongiosa of both areas. (Pearson's silver impregnation method. ×250; reduced ⅗.)

properly vascularized, with normal endochondral ossification. After seven weeks on the deficient diet, the condylar shape became flattened due to the retardation of osteogenesis and chondrogenesis. The internal layer now con-

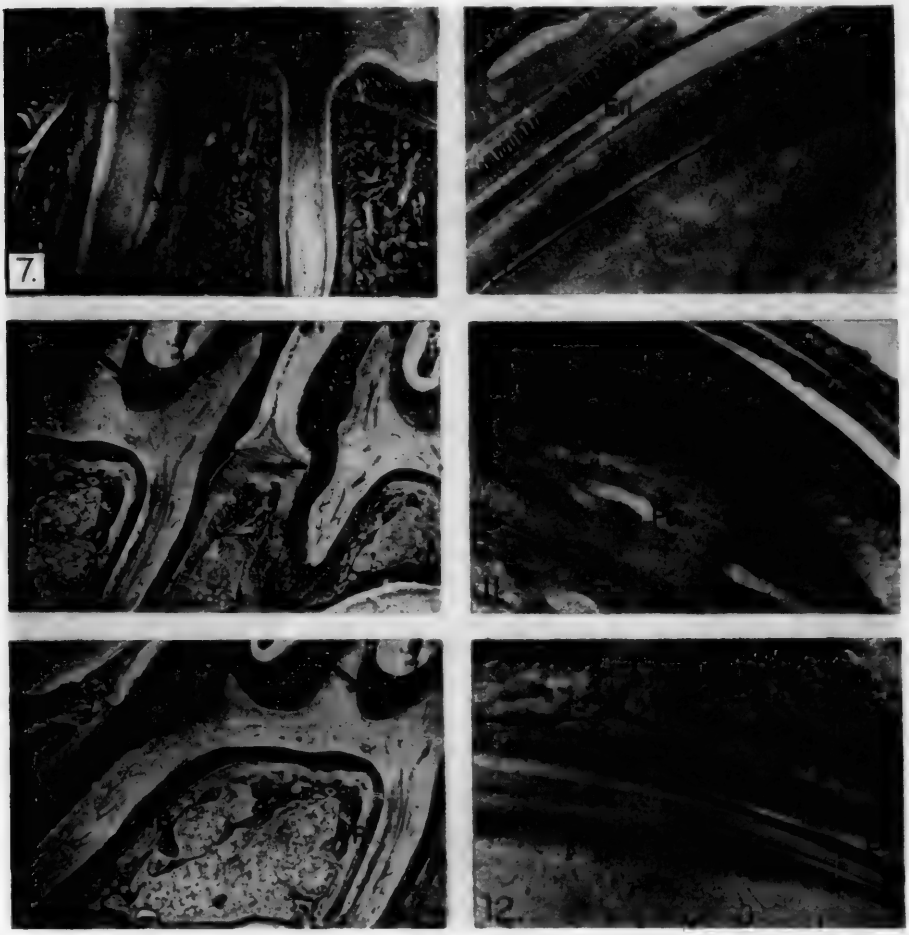


Fig. 7.—Control animal. Interradicular area. Note well-formed alveolar bone with oriented attachment fibers. (Pearson's silver impregnation method. ×400, reduced 3/5.)

Fig. 8.—Experimental animal on deficient diet for three weeks. Interseptal and interradicular areas between molars. Note osteoporosis of alveolar process. (Pearson's silver gelatin impregnation method. ×250; reduced 3/5.)

Fig. 9.—Experimental animal on deficient diet for three weeks. Interradicular area. Note loss of trabeculae in spongiosa and fragmentation of attachment fibers. (×400; reduced 3/5.)

Fig. 10.—Control animal. Incisor tooth. Dentin appears homogenous. (×400; reduced 3/5.)

Fig. 11.—Experimental animal on deficient diet for seven weeks. Incisor tooth. Note increase in number of interglobular areas in the dentin. (Pearson's silver gelatin impregnation method. ×400; reduced 3/5.)

Fig. 12.—Repleted animal. Incisor tooth. Dentin appears homogenous. (Pearson's silver gelatin impregnation method. ×400; reduced 3/5.)

sists of two to three cells' thickness, and apparently no hypertrophic cartilage cells are present. There is an absence of bony spicules in the subchondral area.

Alveolar process: The alveolar process of the molar teeth consists of two layers, the spongiosa and alveolar bone proper. The latter consists of bundle and lamellated bone perforated by blood vessels and nerves. It is lined by osteoblasts, and radiating from it are Sharpey's fibers, the attachment fibers of the periodontal membrane (Fig. 6). This figure also shows the well-developed trabeculations of the spongiosa. Both the radicular and interseptal areas of the alveolar process show marked changes, even after three weeks, which are best demonstrated in the spongiosa (Fig. 8). The crestal area of the interseptal bone does not appear to be affected except for a lacunar-type resorption of bone at its occlusal border, with a concomitant loss of collagenous fibers. There is a disappearance of the spicules, so that only a shell of alveolar bone remains. In the interseptal bone, the crestal area is not involved and the attachment fibers are still evident. In the interradicular area, the attachment fibers show localized areas of both fragmentation and disappearance (Fig. 9).

Incisor tooth: In the control animals the calcified dentin appears as a homogenous structure, stains evenly with hematoxylin and triosin, and shows regular striations with silver impregnation (Fig. 10). The predentin is narrow and even in width. After three weeks on the experimental diet, the incisor tooth appeared unaffected. On the other hand, marked changes are noted in these animals after seven weeks. In Fig. 11, which is a section from the incisor of a seven-week animal, there is marked disturbance in the process of dentinogenesis. Three layers may be seen; the outer and older region of normal dentin is made up of striations, immediately below this the dentin exhibits a hypocalcification, as demonstrated by the increased number of interglobular areas, and below this the predentin is very irregular.

DISCUSSION

This study confirms previous investigations on the essentiality of tryptophane for growth in the rat as well as the importance of its deficiency in the production of such lesions as alopecia and cataract formation. However, the role of this amino acid in the formation of the teeth and supporting structures has been meager. It cannot be stated at the present time whether the dental effects reported are specific lesions characteristic of tryptophane deficiency or the general manifestations of impaired protein metabolism. It remains for future investigations to determine the existence of any possible specificity.

The inhibition and retardation of both endochondral and periosteal bone formation reported in this study may be a direct result of reduced protein synthesis. This is made evident by the definite reduction in the number of cells in the proliferating zone, with the consequent concomitant reduction of calcifying cartilage. Marked osteoporosis was found in both the epiphysis and diaphysis of the femur only after a depletion period of seven weeks, but

regressive changes of this degree were evident in the alveolar process as early as three weeks. At this time the femur had not yet been markedly affected. It appears, therefore, that the alveolar process is much more sensitive to this type of deficiency than the long bones. It might be deduced that the stresses of constant occlusion may have aggravated the deficiency symptoms in this general area.

The pathologic changes were associated with a reduction of the amount of osteoid tissue as well as osteoblastic activity. The reversibility of the pathologic changes was evidenced on examination of the repleted animals. The latter showed definite evidence of renewed osteogenic and chondrogenic activity, and at an accelerated rate.

In contradistinction to Hunter,[2] we were able to induce pathologic changes in the rat incisor. This is undoubtedly due to our use of young growing animals. Since the process of growth can exist only with the predominance of anabolic reactions, it is reasonable to expect that the nutritional support for these biochemical reactions must be much more critical in the young as compared to the adult.

SUMMARY

Inhibition of both endochondral and periosteal bone formation was produced as a result of tryptophane deficiency. Regressive changes were observed at the end of three weeks in both the femur and alveolar process. In contradistinction to results on animals with dietary kidney damages produced by early choline deficiency, the disturbed growth of bone and dental tissue following early tryptophane deficiency can be corrected by subsequent supplementation of the deficient diet.

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THE INDEPENDENCE OF CARIES EXPERIENCE AND SALIVARY TRYPTOPHANE CONTENT

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THIS study was made in order to obtain quantitative data concerning a statement by Turner and Crowell (1947) that "a lack of tryptophane or similar indole-containing molecule shows a striking correlation with the degree of dental decay." They used the Hopkins-Cole reaction, in which the sample is mixed with glyoxylic acid and exposed to a layer of concentrated sulfuric acid; in the presence of tryptophane, or the corresponding amine, a violet coloration is observed at the interface. These authors found a marked reaction in this test with the salivas of all seven individuals without caries. Only one of seventeen individuals with active caries produced saliva in which tryptophane was observed by this test. They gave details of the qualitative results, of their measurements of oxidation-reduction potentials, and of the time taken to decolorize starch-iodide mixtures. It was concluded that saliva from individuals with caries was oxidative in its action on starch, whereas that from normal mouths was reductive.

The tryptophane content of saliva has been examined quantitatively by several authors. Microbiologic assays by Kirch, Kesel, O'Donnell, and Wach (1947) showed 1 to 9 μg available (free) tryptophane in autoclaved saliva (eighteen patients with and without caries). Berg, Burrill, and Fosdick (1946) determined total salivary tryptophane after incubating saliva for one hour under aerobic conditions, in the course of an investigation of the putrefaction of salivary proteins. Calculations from their values for thirty-four cases of varying periodontal conditions show a mean and standard deviation of 22.4 ± 9 μg per milliliter.

METHODS

Saliva Samples Investigated.—The salivary tryptophane levels of ninety individuals, in three groups, were determined in order to establish the normality of a fourth group of fifty-five individuals for which the caries experience was known. The composition of the groups was as follows:

Group I: Twenty-eight dental students.
Group II: Thirty-four school children.
Group III: Twenty-eight student hygienists.
Group IV: Fifty-five dental outpatients.

Stimulated saliva was obtained two hours after breakfast by having the subject chew small slabs of paraffin wax for twenty minutes (rejecting the

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first portion). Volumes collected during this period were recorded (Table I). The samples were stored in the refrigerator under liquid paraffin for not more than two days. Loss of tryptophane was not found to amount to more than 1 per cent for each day's storage under these conditions. In the meantime, Lactobacillus, buffer capacity, and modified Fosdick tests carried out by Dewar (1953) led to an inference as to the caries susceptibility of each patient providing the samples (Group IV). In Table I, S refers to high, marked, or average susceptibility, and N refers to samples whose properties showed that susceptibility was slight.

TABLE I

COMPARATIVE VALUES* FOR DMF COUNTS, FLOW RATES, TRYPTOPHANE CONTENTS, AND SUSCEPTIBILITY GRADINGS OF FIFTY-FIVE SALIVA SAMPLES

DMF COUNT	RATE OF FLOW	TRYPTOPHANE	SUSCEPTIBILITY	DMF COUNT	RATE OF FLOW	TRYPTOPHANE	SUSCEPTIBILITY	DMF COUNT	RATE OF FLOW	TRYPTOPHANE	SUSCEPTIBILITY	DMF COUNT	RATE OF FLOW	TRYPTOPHANE	SUSCEPTIBILITY	DMF COUNT	RATE OF FLOW	TRYPTOPHANE	SUSCEPTIBILITY
0	20	8	N	5	21	22	N	10	13	29	S	14	28	33	S	21	34	18	S
0	44	20	N	7	19	12	N	10	19	28	S	15	34	10	S	21	23	25	S
0	42	31	N	7	26	23	N	11	28	20	S	15	16	36	S	21	24	30	S
1	40	26	N	7	20	25	N	11	28	37	S	16	38	11	S	22	18	19	S
2	28	6	N	8	25	29	S	11	25	38	S	16	17	16	S	22	32	30	S
2	50	8	N	9	30	15	S	12	17	12	S	17	13	19	S	24	38	37	S
2	16	11	N	9	32	17	N	12	37	19	S	17	31	20	S	25	26	28	S
2	34	14	N	9	65	17	N	12	47	35	S	17	11	29	S	26	22	12	S
2	43	26	N	9	26	18	N	12	23	14	S	18	23	25	S	28	53	14	S
3	8	40	N	9	23	18	S	14	38	16	S	20	23	36	S	29	47	29	N
4	29	22	N	10	38	16	S	14	62	29	S	20	20	20	S	30	29	30	S

*DMF counts: arranged in order of magnitude.
Flow rates: volume (milliliters) collected in twenty minutes.
Tryptophane: micrograms per milliliter of saliva.
Susceptibility gradings: S—susceptible and N—not susceptible.

Method of Analysis.—Attempts were made to derive quantitative values for the reaction mixture used by Turner and Crowell. It was necessary to avoid local heating during the addition of sulfuric acid, otherwise destruction of salivary carbohydrate occurred. No charring took place under the conditions suggested by Winkler (1934) in which the reaction mixture was kept cool throughout, but some saliva samples were found to inhibit standards. Nearly linear dose-response curves were obtained when sulfuric acid (3.0 ml.) was mixed gradually with saliva samples (1.0 ml.) and tryptophane (10 to 70 μg per milliliter). However, results were difficult to reproduce by this type of reaction, and another method for estimating total tryptophane was investigated.

Coupling with Ehrlich's reagent in strongly acid solution proved a far more certain procedure for determining tryptophane in saliva. All the analyses reported here were obtained by this procedure as modified by Horn and Jones (1944). A similar method (Boyd, 1929) was adopted by Burrill, Berg, and Fosdick (1946) in their study of periodontal disease. In the process used

here, the centrifuged sample (1.5 ml.) was mixed with p-dimethylaminobenzaldehyde (0.5 ml. of a 5 per cent solution in 11 N hydrochloric acid), and made up to 7.0 ml. with more 11 N acid. The mixture was shaken with 0.2 per cent sodium nitrite (0.05 ml.) after five minutes, and the optical density was read not less than ten minutes later in a photoelectric colorimeter with a red filter (Ilford 204; 90 per cent transmittance at 630 μ). The galvanometer carried a logarithmic scale indicating a 25 per cent reduction in the optical densities of reaction mixtures when 25 μg samples of tryptophane were added to 0.5 ml. of "average" saliva. Before analysis, samples were centrifuged for ten minutes at 2,000 r.p.m. No difference in tryptophane content could be detected if samples were centrifuged for another twenty minutes. Without this clarification, however, values were 15 per cent higher because of the presence of cellular material. When resting saliva was collected, the tryptophane level was not variable in successive 5 ml. portions. A constant tryptophane level was observed in activated saliva after 5 ml. portions had been collected; the first fraction contained 40 per cent more tryptophane than this, and resting saliva 40 per cent less.

The rate of color development depended partly on the quantity measured and partly on the type of saliva. Free tryptophane was indicated by the appearance of a pink tint on first adding the aldehyde, especially with salivas which had a marked odor. When salivas failed to develop a blue color without the addition of nitrite, blank values for the optical density measurements could be obtained; these were about 5 per cent of the average values for the reduced system ten minutes after the nitrite had been added.

The coefficient of variation for the duplicated analyses was below 2 per cent and that for the DMF counts was about 3 per cent. Samples which were obtained on different occasions were much less consistent in tryptophane content, the coefficient of variation being as high as 25 per cent in this case, although the conditions of collection were kept constant.

RESULTS

Table I records data on the salivary tryptophane levels of the individuals in Group IV. Ranges, means, and standard deviations for the four groups are given in Table II. The values for all groups have been classified in Table III to show the abnormal distribution, there being about one-half as many values in the central range as would be expected.

TABLE II

MEANS, RANGES, AND STANDARD DEVIATIONS OF TRYPTOPHANE LEVELS IN FOUR GROUPS OF SALIVA SAMPLES

POPULATION	GROUP	NUMBER OF CASES	TRYPTOPHANE (μG/ML.)		
			RANGE	MEAN	STANDARD DEVIATION
Students	I	28	11-35	23	8
Children	II	34	10-35	23	8
Hygienists	III	28	13-37	23	8
Outpatients	IV	55	6-40	22	9

TABLE III

ABNORMAL FREQUENCY DISTRIBUTION OF TRYPTOPHANE LEVELS AT 5 μG CLASS INTERVALS

Tryptophane (μg/ml.)	6-10	11-15	16-20	21-25	26-30	31-35	36-40
Number of samples	5	19	39	23	38	13	8

The correlation coefficient of 0.10 between DMF counts and salivary tryptophane levels (Group IV) does not account for the low number of values in the central range, an effect which was noticed in all groups. No greater correlation was observed when allowance was made for the volume of saliva collected during the test period, or for the assessment of susceptibility by biochemical tests.

DISCUSSION

It is considered likely that Turner and Crowell did not observe tryptophane in the saliva samples from caries-active patients because of the higher oxidative power of such salivas which they noted at that time. In the present study, the tryptophane level measured in the presence of nitrite was often quite high in those mouths showing the most caries.

Incubation of saliva for one hour under aerobic conditions, as favored in the experiments of Berg, Burrill, and Fosdick (1946), did not appear to affect the tryptophane level, judging from the mean and standard deviation, which are both very similar to those shown in Table II.

The tryptophane levels in saliva are somewhat less variable and about six times lower than the "tyrosine" levels, according to published values for salivary protein. These were reviewed by Gorlin (1948), who added a further series of thirty-five cases, the values being consistent with a normal distribution. The mean value for salivary protein is now calculated to be 2.9 ± 0.6 mg. per milliliter (186 cases). The authors concerned multiplied tyrosine values by a factor (21) which is known to be suitable for salivary proteins.

Although salivary amylase is thought to be inhibited by tryptophane (Turner and Crowell, 1947), it is likely that the amino acid is only thus effective in the free form, which is very much less in quantity (Berg, Burrill, and Fosdick, 1946) than the total as measured in this study. It would appear that most of the tryptophane-containing protein originates from the accessory salivary glands, since Turner and Meyer (1952) found that the tryptophane content of sublingual and submaxillary saliva was less than that of whole saliva, and parotid saliva contained this amino acid in only two cases of fifty examined.

SUMMARY

1. The total salivary tryptophane level in 145 individuals was estimated by reaction with p-dimethylaminobenzaldehyde in strongly acid solution.

2. A mean value of 23 ± 8 (standard deviation) μg per milliliter was observed, the distribution being abnormal in that there were comparatively few central values.

3. No correlation between DMF counts and salivary tryptophane could be proved for the fifty-five cases in the main group, whether or not allowance was made for the present susceptibility or rate of salivary flow.

I am indebted to Gilbert J. Parfitt for suggesting the need for this confirmatory study, for collecting one-half the samples, and for providing the clinical data, which had been required for another investigation; also to Margaret R. Dewar for making the remaining samples available and for giving details of her assessments of caries susceptibility from a number of biochemical tests performed on the samples comprising the main group.

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ENZYMES OF HUMAN SALIVA

I. The Determination, Distribution, and Origin of Whole Saliva Enzymes

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THE enzymatic hydrolysis of synthetic substrates whose end products are capable of forming dyes has been used for quantitative histochemical and physiologic fluid determinations. These have been employed to study the effects of local and systemic disturbances,[1-8] and may be utilized in evaluating the role of enzymes in disease processes manifesting themselves in the oral cavity.

Numerous enzymes have been previously demonstrated in saliva. They include amylase, invertase, maltase, carbonic anhydrase, urease, oxidases, catalase, proteolytic enzymes, lipase, phosphatases, lysozyme, and hyaluronidase.[9-20] While phosphatase, amylase, and lysozyme have aroused the most interest of the enzymes reported to be in saliva, no agreement has been reached concerning their role in various pathologic conditions of the oral cavity.

Enzymes in the oral cavity may originate from several sources: the major and minor salivary glands, bacteria, oral tissues, and ingested substances. It is of primary importance, therefore, to have some fundamental knowledge concerning the various sources of the enzymes present. An attempt toward achieving this end is presented.

All determinations of the ten enzymes included in this study were carried out simultaneously on the same saliva sample obtained from each individual. This approach was used to permit the evaluation of the possible interrelationship between individual enzymes. Thus, the determination, distribution, and origin of salivary enzymes could afford a better understanding of the factors distinguishing the normal from the abnormal in oral conditions.

First, the presence of certain hydrolytic enzymes in whole saliva was tested. Once their presence was established, whole saliva samples were obtained from 56 persons, and the range of activity and the mean activity of these enzymes were determined. Next, cannulated parotid saliva was taken and tested for the presence of these enzymes; mean and range of activity were also determined. Finally, inocula of whole saliva were incubated in beef brain-heart infusion broth to establish qualitatively whether or not the flora of the oral cavity was capable of elaborating these enzymes.

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TABLE I

ENZYME	VOLUME OF SALIVA (ML.)	SUBSTRATE	SUBSTRATE CONCENTRATION (FINAL) (MG. PER C.C.)	pH	BUFFER	PERIOD OF INCUBATION AT 37° C. (HOURS)	DIAZONIUM SALT CONCENTRATION (MG. PER C.C.)	TRICHLOROACETIC ACID CONCENTRATION	EXTRACTION AGENT
Acid phosphatase	0.05	Monosodium β-naphthyl-phosphate	0.1	4.8	0.2 M acetate	2.0	4.0	40% (1.0 c.c.)	Ethyl acetate
Alkaline phosphatase	1.00	Monosodium β-naphthyl-phosphate	0.1	9.1	0.1 M veronal	1.0	4.0	40% (1.0 c.c.)	Ethyl acetate
Esterases (total)	0.05	β-Naphthyl-acetate	0.01	7.4	0.1 M veronal	1.0	4.0	40% (1.0 c.c.)	Ethyl acetate
Cholinesterase	3.00	β-Carbonaphthoxycholine iodide	0.108	7.4	0.1 M veronal	1.0	2.0	80% (1.0 c.c.)	Ethyl acetate
Lipase	0.10	β-Naphthyl-laurate	0.071	7.4	0.1 M veronal	5.0	4.0	40% (1.0 c.c.)	Ethyl acetate
Sulfatase (aryl)	2.00	Potassium 6-benzoyl-2-naphthylsulfate	0.25	6.1	0.1 M acetate	24.0	1.0	40% (1.0 c.c.)	Ethyl acetate
β-D-galactosidase	1.00	6-Bromo-2-naphthyl-β-D-galacto-pyranoside	0.15	5.0	0.1 M acetate	2.0	1.0	80% (2.0 c.c.)	Chloroform
β-glucuronidase	3.00	Sodium 8-benzoyl amino 2-naphthyl glucuronide	0.20	5.0	0.1 M acetate	4.0	1.0	80% (1.0 c.c.)	Ethyl acetate

The enzymes measured were acid phosphatase, alkaline phosphatase, total esterase, cholinesterase, lipase, aryl-sulfatase, beta-D-galactosidase, beta-glucuronidase, hyaluronidase, and lysozyme.

PROCEDURE

The subjects were given code numbers, and 25 ml. of early-morning paraffin-stimulated saliva was collected in sterile bottles from each individual. The salivas were obtained immediately on arising, before brushing of the teeth, eating or rinsing of the mouth. All enzyme activities were assayed from fresh saliva specimens with the exception of beta-D-galactosidase and beta-glucuronidase, which were determined on stored frozen samples (−10° C. for ten days). The final volume of saliva used for each colorimetric test system is given in Table I. These variations were necessary in order to bring the quantity of dye released from the substrates during incubation within the range of the colorimeter.

Hyaluronidase activities of the saliva samples were determined by a modification of viscosimetric method.[21] Lysozyme activities were measured turbidimetrically.[22] The remaining enzyme activities were determined by the colorimetric methods of Seligman and his co-workers,[23-27] modified for use with saliva. The chromogenic hydrolysis product was either beta-naphthol or a substituted naphthol. When combined with tetrazotized diorthoanisidine, these produced a water-insoluble dye which was extracted with ethyl acetate or chloroform and measured photocolorimetrically (Klett-Summerson).

The substrate solution (1.0 ml. for sulfatase, 5.0 ml. of the others) was mixed with the required saliva dilutions in a 20 ml. test tube and incubated at 37° C. for the time periods indicated in Table I. After incubation, the pH was adjusted with alkaline salt solutions[23, 27] to bring the pH to the optimum for coupling with the diazonium salt. One milliliter of freshly prepared solution of tetrazotized diorthoanisidine was added to each tube. After three to fifteen minutes, a trichloroacetic acid solution was added to facilitate extraction of the dye from the protein complex and to prevent decomposition of the excess diazonium salt (Table I). The dye was extracted by the addition of 10.0 ml. of either ethyl acetate or chloroform (Table I). The tubes were then thoroughly shaken to extract the dye. The resulting emulsion was broken by centrifugation at 1,500 r.p.m. for fifteen minutes. The colored organic layer was transferred with a pipette to a Klett tube for measurement of its color density with a photoelectric colorimeter at 540 m.

The method of Rutenburg, Cohen, and Seligman[26] was adapted for the determination of aryl-sulfatase activity of saliva. Twenty-five milligrams of the sulfatase substrate (6-benzoyl-2-naphthyl sulfate)* was dissolved in 100 ml. of acetate buffer, pH 6.1 (0.1 M). Two cubic centimeters of whole saliva was added to each tube containing 1 c.c. of the substrate solution. The tubes were shaken and incubated at 37° C. for twenty-four hours. Upon removal from the incubator, 1 c.c. of 0.2 M sodium dihydrogen phosphate was added to each tube. A freshly prepared solution of diazo blue B (1 c.c. of a 1 mg. per cubic centimeter solution)

*The 6-benzoyl-2-naphthyl sulfate and 8-amino-2-naphthyl glucuronide were obtained through the courtesy of Dr. Arnold M. Seligman, Beth Israel Hospital, Boston, Mass.

was added, and the tubes were shaken and allowed to stand at room temperature for fifteen minutes to permit complete color development. One cubic centimeter of 40 per cent trichloroacetic acid solution was added, and the color (dye) was extracted with 10 ml. of ethyl acetate, as described in the previous procedures.

Color density was converted to micrograms of beta-naphthol or substituted beta-naphthol from standard curves. The curves were prepared by adding varying amounts of these compounds to pooled saliva followed by immediate color development with diazo blue B.

One unit of activity is defined as the amount of enzyme which liberates the color equivalent of 10 mg. of beta-naphthol or substituted beta-naphthol during the period of incubation at 37° C. This unit was used to facilitate comparison of oral levels of the various enzymes.

The hyaluronidase activity is expressed as the percentage of the half time, that is, the time required to achieve a 50 per cent decrease in viscosity of the substrate in forty-five minutes at 25° C.[21]

The procedure for the determination of beta-glucuronidase activity consisted of first dissolving 20.0 mg. of 8-amino-benzoyl-2-naphthyl glucuronide in 80 ml. of distilled water. This was diluted to 100 ml. with acetate buffer (0.1 M), pH 5.0. Five milliliters of this substrate solution was mixed with 3.0 ml. of whole saliva, and the mixture was incubated at 37° C. for four hours. After removal from the incubator, 0.5 ml. of 0.2 M trisodium phosphate solution was placed in each tube. One cubic centimeter of a 1 mg. per cubic centimeter solution of diazo blue B was added, and five minutes was allowed for color development. One cubic centimeter of 80 per cent trichloroacetic acid was used to stop the reaction. The dye was extracted with 10.0 ml. of ethyl acetate, and the Klett readings were taken.

The lysozyme activity of saliva was determined by comparison with a standard solution containing 1/100,000 of crystalline egg white lysozyme (Delta) which had a reaction time of 1.25 minutes in 0.85 per cent NaCl at pH 6.8. The units reported were calculated by dividing the reaction time of the individual salivas by the reaction time of the standard (1.25), and multiplying the reciprocal of this value by fifty. Since the quantity of saliva tested was 2.0 ml., this expressed the activity as the amount found in 100 ml. of saliva.

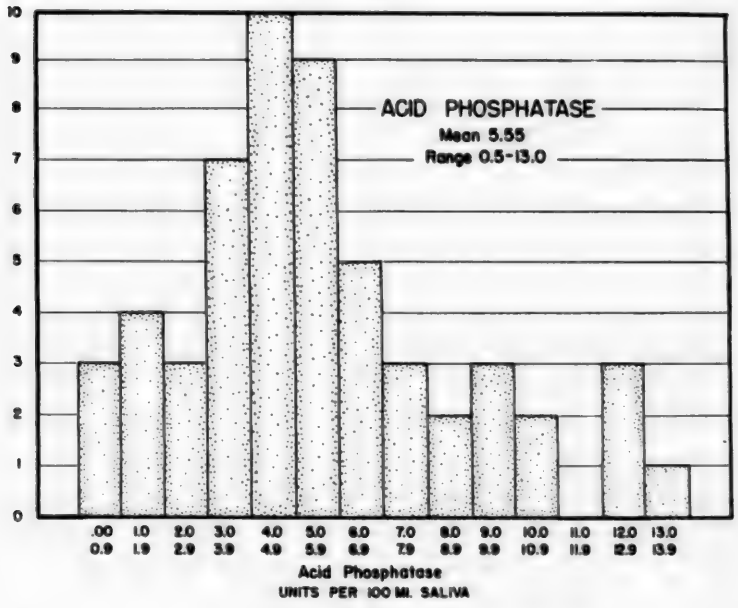
RESULTS

Enzyme Activity of Whole Saliva.—

Phosphatases: The salivas of 56 adults contained a mean acid phosphatase activity of 5.55 units per 100 ml. of saliva, with a range of 0.5 to 13.0 units per 100 ml. (Fig. 1). The saliva of these same persons contained a mean of 0.478 unit of alkaline phosphatase activity per 100 ml., with a range of 0.025 to 1.11 units per 100 ml. of saliva (Fig. 2).

Esterases, serum cholinesterase, lipase: The mean total esterase activity of the saliva samples studied was 3.23 units per 100 ml., with a range of 0.10 to 7.50 units per 100 ml. (Fig. 3). Of the 56 salivas tested, 16 had no demonstrable cholinesterase activity. The range of cholinesterase activity was from 0.000 to 0.221 unit per 100 ml., with a mean activity of 0.053 unit (Fig. 4). The lipase activity varied from 0.20 to 2.70 units per 100 ml., the mean activity being 1.18 units per 100 ml. of saliva (Fig. 5).

Fig. 1.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Mean 5.55
Range 0.5-13.0
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1
2
3
4
5
6
7
8
9
10
.00 0.9
1.0 1.9
2.0 2.9
3.0 3.9
4.0 4.9
5.0 5.9
6.0 6.9
7.0 7.9
8.0 8.9
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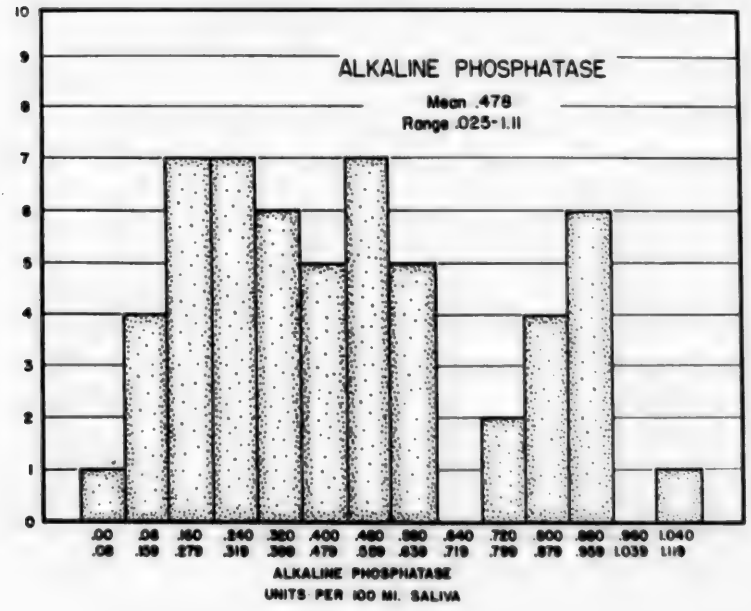
Acid Phosphatase
UNITS PER 100 ML. SALIVA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Mean .478
Range .025-1.11
0
1
2
3
4
5
6
7
8
9
10
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.08 .159
.160 .279
.240 .319
.320 .399
.400 .479
.480 .559
.560 .639
.640 .719
.720 .799
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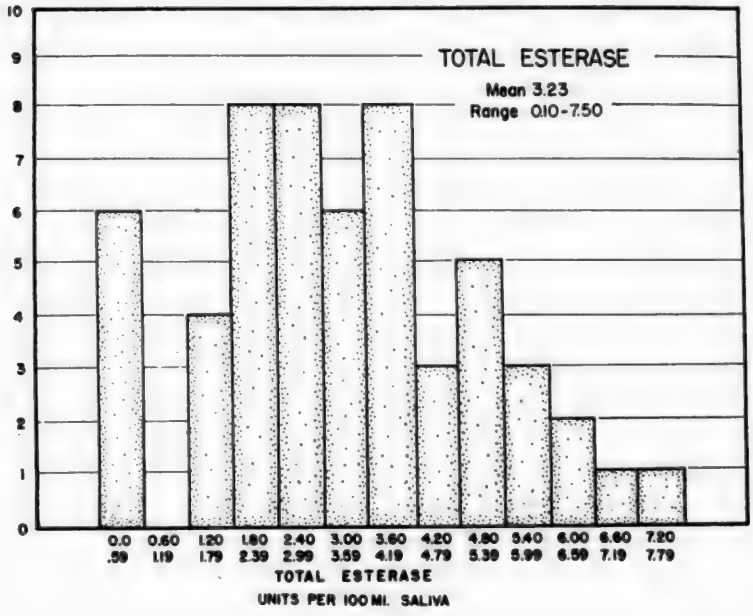
1.040 1.119
ALKALINE PHOSPHATASE
UNITS PER 100 ML. SALIVA

Fig. 2.

Fig. 3.


TOTAL ESTERASE
Mean 3.23
Range 0.10-7.50
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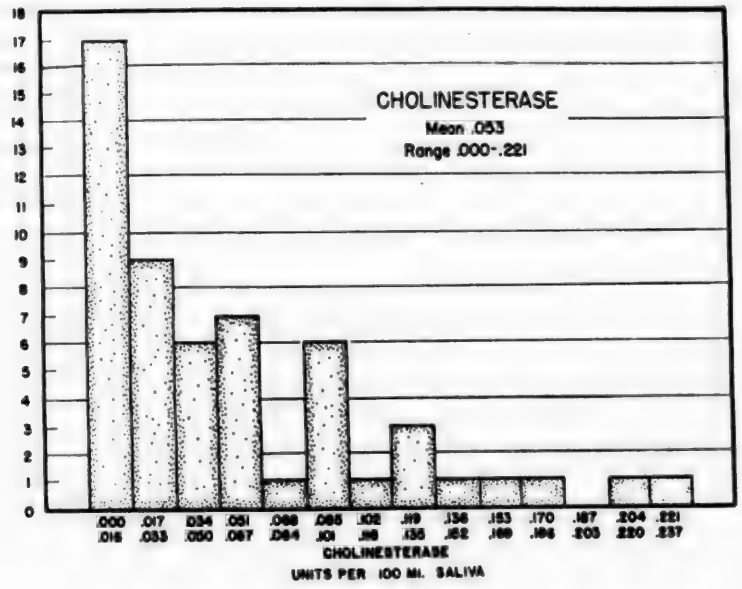
0.60 1.19
1.20 1.79
1.80 2.39
2.40 2.99
3.00 3.59
3.60 4.19
4.20 4.79
4.80 5.39
5.40 5.99
6.00 6.59
6.60 7.19
7.20 7.79
TOTAL ESTERASE
UNITS PER 100 ML. SALIVA


CHOLINESTERASE
Mean .053
Range .000-.221
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
.000 .016
.017 .033
.034 .050
.051 .067
.068 .084
.085 .101
.102 .118
.119 .135
.136 .152
.153 .169
.170 .186
.187 .203
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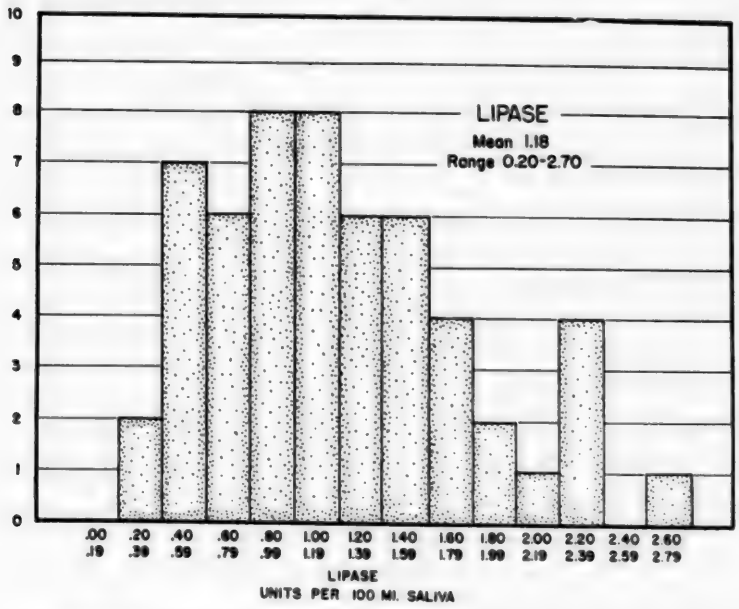
.221 .237
CHOLINESTERASE
UNITS PER 100 ML. SALIVA

Fig. 4.

Fig. 5.


LIPASE
Mean 1.18
Range 0.20-2.70
10
9
8
7
6
5
4
3
2
1
0
.00 .19
.20 .39
.40 .59
.60 .79
.80 .99
1.00 1.19
1.20 1.39
1.40 1.59
1.60 1.79
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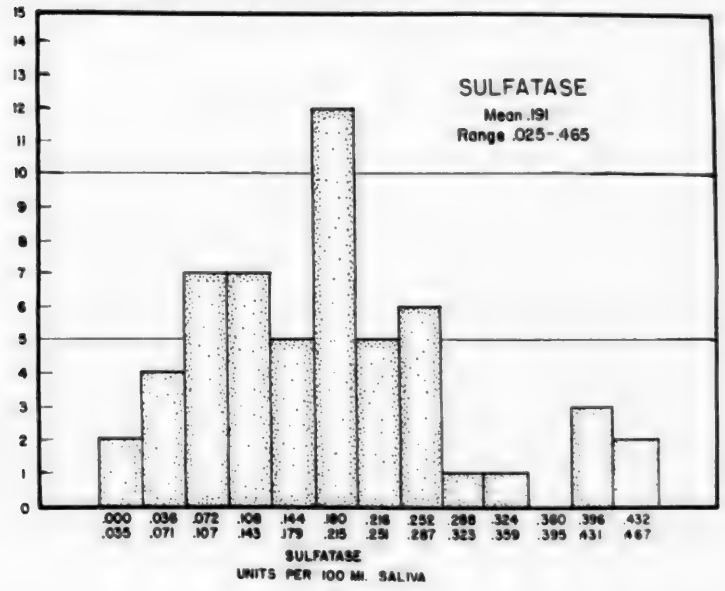
2.40 2.59
2.60 2.79
LIPASE
UNITS PER 100 ML. SALIVA


SULFATASE
Mean .191
Range .025-.465
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
.000 .035
.036 .071
.072 .107
.108 .143
.144 .179
.180 .215
.216 .251
.252 .287
.288 .323
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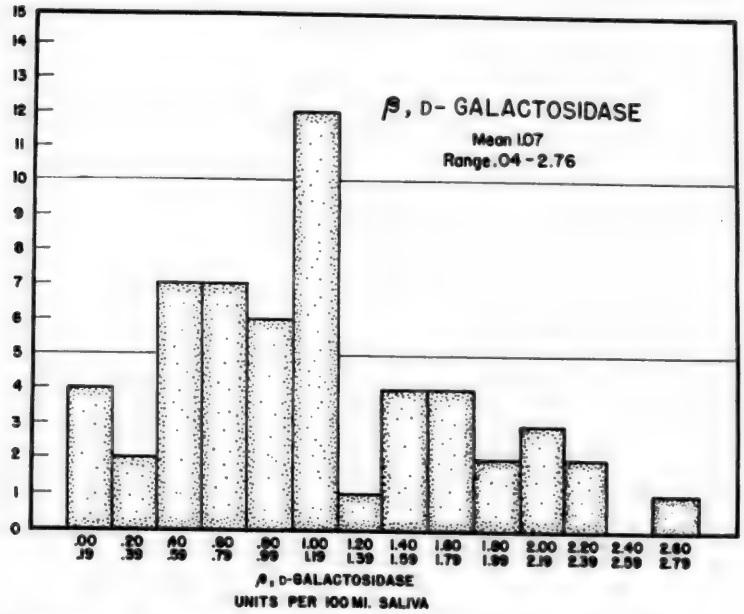
.360 .395
.396 .431
.432 .467
SULFATASE
UNITS PER 100 ML. SALIVA

Fig. 6.

Fig. 7.


β, D- GALACTOSIDASE
Mean 1.07
Range .04 - 2.76
.00 .19
.20 .39
.40 .59
.60 .79
.80 .99
1.00 1.19
1.20 1.39
1.40 1.59
1.60 1.79
1.80 1.99
2.00 2.19
2.20 2.39
2.40 2.59
2.60 2.79
β, D-GALACTOSIDASE
UNITS PER 100 ML. SALIVA

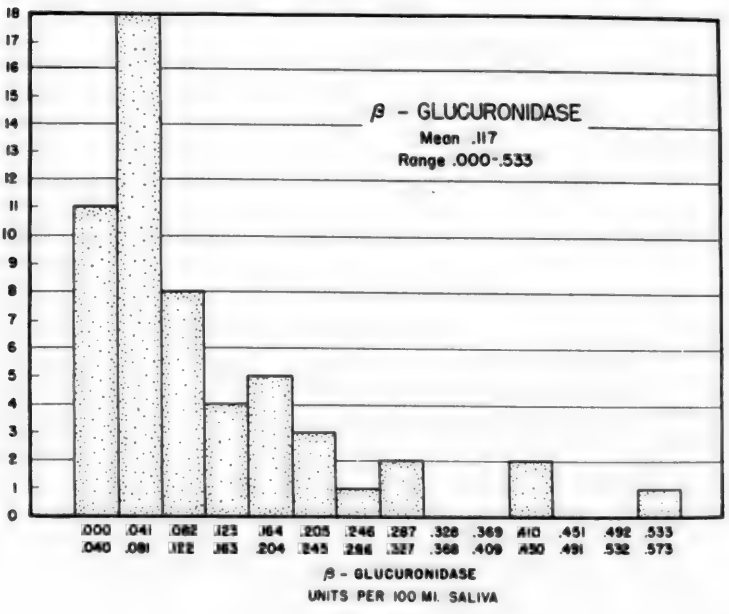
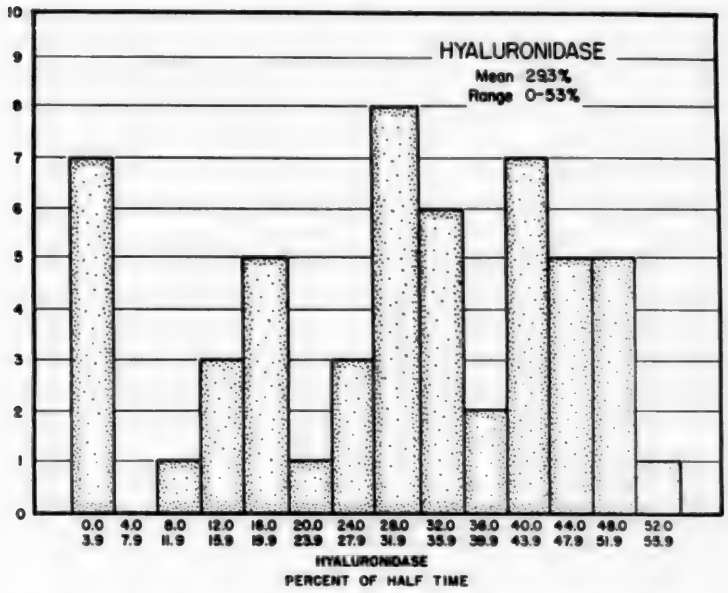

β - GLUCURONIDASE
Mean .117
Range .000-.533
.000 .040
.041 .081
.082 .122
.123 .163
.164 .204
.205 .245
.246 .286
.287 .327
.328 .368
.369 .409
.410 .450
.451 .491
.492 .532
.533 .573
β - GLUCURONIDASE
UNITS PER 100 ML. SALIVA

Fig. 8.

Fig. 9.


HYALURONIDASE
Mean 29.3%
Range 0-53%
10
9
8
7
6
5
4
3
2
1
0
0.0 3.9
4.0 7.9
8.0 11.9
12.0 15.9
16.0 19.9
20.0 23.9
24.0 27.9
28.0 31.9
32.0 35.9
36.0 39.9
40.0 43.9
44.0 47.9
48.0 51.9
52.0 55.9
HYALURONIDASE
PERCENT OF HALF TIME

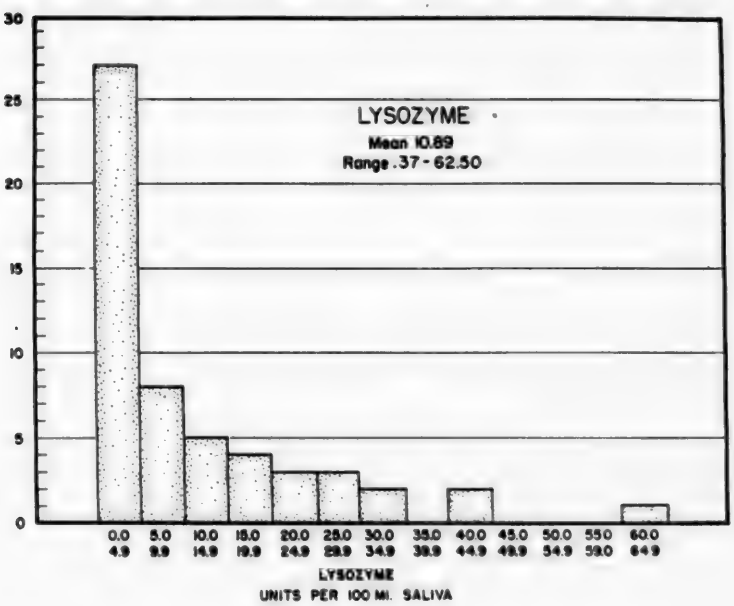

LYSOZYME
Mean 10.89
Range .37 - 62.50
30
25
20
15
10
5
0
0.0 4.9
5.0 9.9
10.0 14.9
15.0 19.9
20.0 24.9
25.0 29.9
30.0 34.9
35.0 39.9
40.0 44.9
45.0 49.9
50.0 54.9
55.0 59.0
60.0 64.9
LYSOZYME
UNITS PER 100 ML. SALIVA

Fig. 10.

Sulfatase, beta-D-galactosidase, beta-glucuronidase: The range of arylsulfatase activity was from 0.025 to 0.465 unit per 100 ml., and had a mean activity of 0.191 unit per 100 ml. (Fig. 6). Beta-D-galactosidase activity of frozen saliva was found to have a range of 0.04 to 2.76 units per 100 ml., with a mean of 1.07 units per 100 ml. (Fig. 7). In the determination of the beta-glucuronidase activity, three persons were found to have no detectable activity in their saliva. The mean activity was determined to be 0.117 unit per 100 ml., with a range of 0.000 to 0.533 unit per 100 ml. of frozen saliva (Fig. 8).

Mucopolysaccharidase activity: Of the saliva samples studied, 7 persons were found to have no hyaluronidase activity. Two of these were among the 3 in whom no beta-glucuronidase could be demonstrated, while 6 of the 7 were in the group showing no cholinesterase activity. The mean hyaluronidase activity was 29.3 per cent, with a range of 0.0 per cent to 53.0 per cent (Fig. 9). The lysozyme activity was found to be extremely variable. This activity ranged from 0.37 to 62.50 units per 100 ml., with an average activity of 10.89 units per 100 ml. of saliva. However, almost one-half the subjects tested fell in the median range, 0.00 to 4.90 units per 100 ml. (Fig. 10).

TABLE II

ENZYME	RANGE OF ACTIVITY	MEAN ACTIVITY	SOURCE		
			WHOLE SALIVA	PAROTID SALIVA	ORAL BACTERIA
Acid phosphatase	0.5-13.0	5.55	+	+	+
Alkaline phosphatase	0.025-1.11	0.478	+		+
Total esterases	0.10-7.50	3.23	+	+	+
Cholinesterase	0.000-0.221	0.053	+	+	+
Lipase	0.20-2.70	1.18	+	+	+
Sulfatase	0.025-0.465	0.191	+		?
β-D-Galactosidase	0.04-2.76	1.07	+		+
β-Glucuronidase	0.000-0.533	0.117	+	+	+
Lysozyme	0.37-62.50	10.89	+	+	
Hyaluronidase*	0.0%-53.0%	29.3%	+		+

*Activities for all enzymes except hyaluronidase are expressed as units per 100 ml. of saliva.

Parotid Saliva.—Sterile parotid saliva, obtained by cannulation with a parotid cup (Curby[42]), was shown to contain acid phosphatase, esterases, cholinesterase, lipase, beta-glucuronidase, and lysozyme. Sterility was verified by plating an aliquot on horse blood agar plates.

A random sampling of 10 persons from the original group showed that the *acid phosphatase* activity varied from 0.25 to 0.77 unit per 100 ml. of parotid saliva, with a mean activity of 0.423 unit. The mean *total esterase* activity was 0.034 unit per 100 ml., with a range of 0.012 to 0.065 unit per 100 ml. *Cholinesterase* activity ranged from 0.023 to 0.043 unit per 100 ml., with a mean activity of 0.033 unit per 100 ml. *Lipase* activity was found to have a mean activity of 0.142 unit, and ranged from 0.025 to 0.258 unit per 100 ml. *Lysozyme* was found in high concentration in parotid saliva. It ranged from 25 to 136 units per 100 ml., while the mean activity was 67 units. While we could find no evidence of *beta-glucuronidase* activity in parotid saliva, samples tested for us in Dr. William Fishman's laboratory exhibited the presence of an enzyme capable of hydrolyzing phenolphthalein glu-

curonide. The random sampling demonstrated that the parotid activity ranged from 17 to 175 units (Fishman) per 100 ml. of parotid saliva.

In the studies on parotid saliva, no demonstrable activity was found for alkaline phosphatase, aryl-sulfatase, beta-D-galactosidase, and hyaluronidase either in individual samples or in pooled parotid saliva which had been lyophilized and reconstituted at three times normal concentration.

Oral Enzymes of Bacterial Origin.—In order to determine whether the difference in the enzyme activity of whole and parotid saliva could be attributed to the oral flora, inocula of whole saliva from each of 16 subjects were placed in beef brain-heart infusion broth (Difco), and incubated for twenty-four hours at 37° C. Using sterile incubated broth as a control, the broth cultures were tested for enzyme activity in the same manner as whole saliva. These results indicated that the microorganisms found in saliva were capable of producing acid phosphatase, alkaline phosphatase, esterases, cholinesterase, lipase, beta-D-galactosidase, beta-glucuronidase, and hyaluronidase.

Effect of Freezing on Salivary Enzymes.—Comparative measurements using frozen (−10° C.) saliva demonstrated decreases in activity for cholinesterase (10 per cent), lipase (10 to 25 per cent), esterase (0 to 15 per cent), aryl-sulfatase (15 to 35 per cent), and beta-glucuronidase (50 per cent). No change was observed for beta-D-galactosidase and lysozyme, but an increase of 40 to 50 per cent was found in acid phosphatase activity and 10 to 20 per cent in hyaluronidase activity. Alkaline phosphatase demonstrated unexplained variable activity. Repeated determinations showed increases to 35 per cent in certain salivas, while others showed consistent decreases of 20 per cent. Variations of less than 10 per cent were within the range of reproducibility of the technics employed.

DISCUSSION

The presence and origin of certain of the salivary enzymes is still controversial. Lura[28] stated that acid and alkaline phosphatase are present in both whole and parotid saliva, while Fitzgerald[29] stated that saliva per se contains little or no phosphatase activity, but that it originates in the bacterial and cellular debris present in saliva. Our data have demonstrated both acid and alkaline phosphatase in whole saliva, while repeated determinations on parotid saliva showed no evidence of alkaline phosphatase activity. The acid phosphatase activity of parotid saliva was approximately 10 per cent of that found in whole saliva, indicating that most of this enzyme is either of exogenous origin or is due to the secretions of the other glands.

Gal and Adler[30] were unable to demonstrate the presence of either cholinesterase or a cholinesterase inhibitor in saliva. The determinations were made on whole and filtered saliva, but no account was made of bacterial contributions. Ord and Thompson,[31] in measuring the rate of hydrolysis of acetylcholine, acetyl-beta-methylcholine, and benzoylcholine by different tissues of the rat, found that salivary glands contained approximately equal proportions of true and pseudocholinesterase. Koelle[32] found both cholinesterase types in the peripheral autonomic ganglia of the parotid gland. Bacteria have also been noted to possess cholinesterase activity.[33] Cholinesterase

activity was found in whole saliva, parotid saliva, and in broth cultures of the mixed flora obtained from the oral cavity. However, the parotid cholinesterase titer represented 60 per cent of the activity of whole saliva. Oral esterases (total) were found to be of both glandular and bacterial origin, with the parotid gland activity contributing about 1 per cent. The lipase activity of parotid saliva amounted to about 10 per cent of the whole saliva lipase titer. This enzyme was also produced by the oral bacteria.

Sulfatase, an enzyme which Pincus[34] has implicated as a causative factor in dental caries, was found in whole saliva, but not in the parotid secretion or in the oral microorganisms by our method. Since mammalian tissues contain aryl-sulfatase, it is possible that its presence is due either to the cellular debris in saliva or to the other glandular secretions. However, it has been indicated that certain bacteria can elaborate these enzymes.[35]

Beta-D-galactosidase activity has been found in certain plants, molds, and mammalian tissues, as well as in various strains of bacteria *(Lactobacillus delbrueckii, Escherichia coli)*.[36] This enzyme, although found in whole saliva, did not appear to be present in parotid saliva. The bacteria inhabiting the oral cavity were found capable of producing this enzyme, and because of their high activity, may be responsible for most of its production.

Fishman, Springer, and Brinette[37] found beta-glucuronidase activity in saliva and suggested that it was secreted by the glandular epithelium. Becker and Friedenwald,[38] from a histochemical study of beta-glucuronidase, found it to be present throughout the entire submaxillary gland, and present only in the tubular epithelium of the sublingual glands. Our data indicate that the parotid activity of this enzyme was from 5 to 20 per cent of the amount found in whole saliva. Organisms have been isolated from the oral cavity which are capable of producing this enzyme.[39] There appeared to be a difference in the type as well as the amount of activity of enzymes from both sources. The parotid enzyme did not appear to be capable of hydrolyzing the substrate employed. However, it was hydrolyzed by whole saliva to a greater degree than was phenolphthalein glucuronide. Some of the bacteria of whole saliva were found to be capable of producing a beta-glucuronidase which hydrolyzed both the phenolphthalein glucuronide and 8-amino-benzoyl-2-naphthyl glucuronide. These preliminary experiments show that there may be a difference in the action of this enzyme, depending on its source, glandular or bacterial, and that this action may be selective.

Skrotskii, Makhlinovskii, and Slutskaia[40] have shown lysozyme to be present in the saliva of 95 to 98 per cent of 336 patients, without any relation to the condition of the mouth. Our study on 56 persons showed that all the saliva samples contained lysozyme, although to a highly varying degree. However, the activity of parotid saliva was considerably higher than that found in whole saliva. This may be due to adsorption of the enzyme on mucin, whose content is relatively higher in whole saliva, and may also be caused in part by the inhibitory effect of certain inorganic ions, such as chloride.[41]

Hyaluronidase, present in whole saliva, but absent from parotid secretion, is evidently of bacterial origin. Mahler and Lisanti[21] have isolated four types of hyaluronidase-producing streptococci from the oral cavity.

The measurement of a spectrum of enzymes under the described conditions has afforded comparisons of the contributions of the activities from glandular and bacterial sources, as well as the total obtained in the admixed (native) state. The secretions of some of the remaining glands, presently under investigation, will aid materially in identifying the more exact sources of the enzymes. The facility of simultaneous measurements of these activities in single salivary samples allows for detection of significant simple or multiple variations.

SUMMARY

1. The ranges, means, and sources of ten enzymes found in native saliva have been determined: acid phosphatase, alkaline phosphatase, total esterases, cholinesterase, lipase, sulfatase, beta-glucuronidase, beta-D-galactosidase, lysozyme, and hyaluronidase.

2. The presence of six of these enzymes was established in parotid saliva: acid phosphatase, total esterases, cholinesterase, lipase, beta-glucuronidase, and lysozyme.

3. Broth cultures used for this study with whole saliva indicated that all but sulfatase and lysozyme were produced by the oral flora.

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PHOSPHATASE IN HUMAN SALIVA: ITS RELATIONSHIP TO CALCULUS AND LACTOBACILLUS COUNTS

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INTRODUCTION

BOTH the origin and function of phosphatase in saliva are still in question. Several sources for this enzyme, including desquamated epithelial cells,[1] gingival tissue,[2, 3] and microorganisms of the saliva,[4-8] have been postulated, the latter being the most probable.

Adamson[1] and Smith[2] were among the first to propose that phosphatase, which is capable of liberating inorganic phosphate from organic phosphate may play a role in the formation of calculus. Glock, Murray, and Pincus[4] and Ennever and Warner[9] found that the organisms most commonly associated with calculus, namely, Actinomyces, show marked phosphatase activity. Tenenbaum and Karshan[10] showed that, in general, people with calculus have higher Ca and P levels in the saliva, and hence they hold tenable the theory that phosphatase plays a role in calculus formation. Saltzmann,[11] on the other hand, using a limited number of patients (fourteen), found that the degree of phosphatase in saliva was not related to the presence and quantity of calculus in the mouth.

The relation of phosphatase to caries activity has also been studied. Lactobacilli, generally associated with the caries process, have been found to possess some phosphatase activity, although of a very low order.[7, 8, 12] Rae[13] reported no apparent relationship between the phosphatase content of saliva and the susceptibility to caries in thirty-nine boys; however, Fitzgerald,[8] in an initial trial with thirty cases, claimed to have obtained generally parallel results between acid phosphatase activity and lactobacillus counts, and he suggested further study of acid phosphatase activity of saliva as an additional laboratory test for caries activity.

The purpose of this investigation was to study simultaneously the relationship of phosphatase levels to the presence of calculus and Lactobacillus counts in the same individuals.

EXPERIMENTAL

Paraffin-stimulated saliva samples were collected from forty-nine senior dental students. Twenty-four had a history of recurrent calculus, and twenty-five were relatively free of calculus. The majority of the students of both groups had had a prophylaxis within one year, and no oral examination was made. The conditions under which the saliva samples were collected and handled, as well as the volume of sample collected, were kept constant as far as possible. In all cases the saliva samples were run within a few hours of collection.

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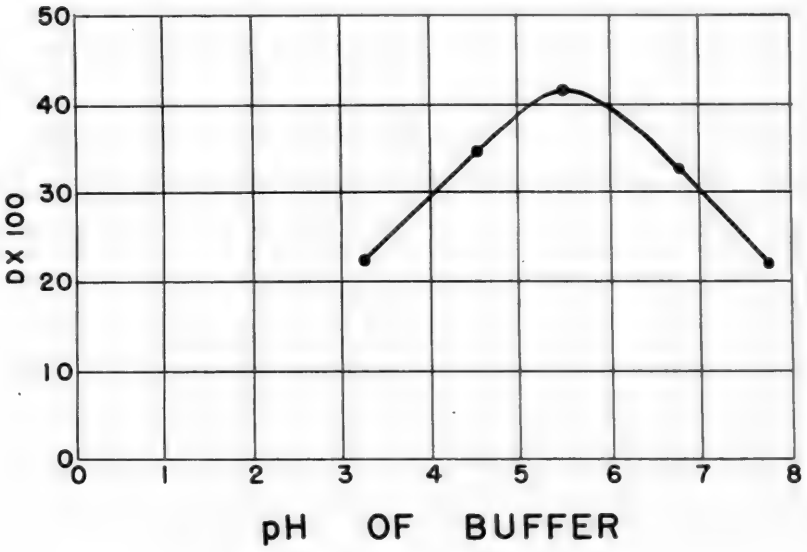
Phosphatase in saliva was determined essentially by the King-Armstrong method, published by Eastman Kodak Company,[14] employing phenyl dihydrogen phosphate disodium salt as a substrate; however, the pH of the acid buffer used was 5.5, density was read using 1 c.c. of filtrate in an Evelyn colorimeter and using a 690 m. filter, and the substrate concentration used was sixteen times that advocated in the method. The general properties of the enzyme were studied prior to the testing of the saliva samples.

Lactobacillus counts were run on aliquots of the saliva samples by the method of Rogosa, Mitchell, and Wiseman.[15] The range of the counts was expressed as 0-4+.*

FIGURE 1

ACTIVITY vs pH



RESULTS

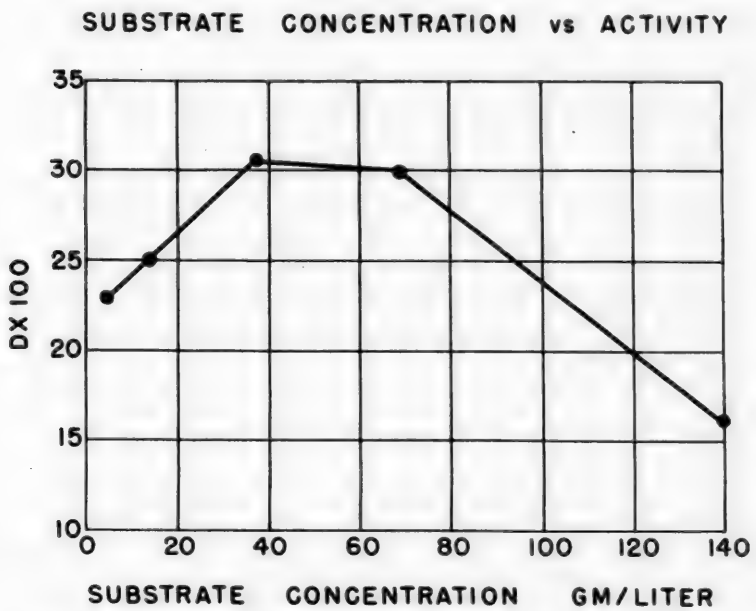
Properties of Salivary Phosphatase.—The maximum activity of the enzyme occurred at pH 5.5, with decreasing activity on either side in the range of pH 3.4-10.9. Using the substrate phenyl dihydrogen phosphate disodium salt, it appears that only an acid phosphatase having an optimum activity at pH 5.5 is found in saliva (Fig. 1). At pH levels greater than shown, activity of the enzyme decreased further. Citrate and bicarbonate buffers were used in the acid and alkaline phosphatase determinations, respectively. Substrate concentration seemed to have a marked effect on enzyme activity, and the optimum

*Number of lactobacilli per cubic centimeter of saliva: ±, to 300; +, to 1,000; ++, to 10,000; +++, to 50,000; ++++, over 50,000.

substrate concentration in the system used was found to be sixteen times that advocated in the Eastman method: that is, 2 c.c. of a solution containing 34.88 Gm. per liter of the substrate were used instead of 2 c.c. containing 2.18 Gm. per liter (Fig. 2). After centrifugation of a sample of saliva, the supernatant contained only 36 per cent of the original activity of the whole saliva. These results are based on whole saliva of one of us (E. Z. H.).

Phosphatase Levels in Students With and Without Recurrent Calculus.—There was no significant difference between the mean salivary phosphatase levels of students with and without recurrent calculus. The mean ± standard error was 0.840 ± 0.097 for the former and 1.014 ± 0.122 for the latter. The values are in terms of milligrams of phenol released per cubic centimeter of saliva per hour.

FIGURE 2



Lactobacillus Counts Versus Phosphatase Levels.—No association of any kind was found between the phosphatase values and Lactobacillus counts in the forty-nine students. The eta correlation ratio was computed for these data and was not found to be significant. The mean Lactobacillus counts for the two groups (with and without calculus) were also not significantly different.

DISCUSSION

Although most of the previous workers spoke of both an alkaline and an acid phosphatase enzyme, in the saliva, the data herein reported show the presence of only one enzyme, with a pH optimum at 5.5.

Using three times as many cases as Saltzmann,[11] we were able to confirm his results showing that the phosphatase levels in persons with and without calculus are not different.

Contrary to the work of Fitzgerald,[8] we were unable to find a significant (at the 0.01 level) relationship between Lactobacillus counts and phosphatase values. Furthermore, when the chi square (X^2) test for association is applied to Fitzgerald's data, no significant relationship is found between these two values at the 0.01 level.

CONCLUSIONS

1. No significant difference was found in the phosphatase levels or Lactobacillus counts of forty-nine students with and without recurrent calculus.

2. No association between the phosphatase levels and Lactobacillus counts was found in these students.

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THE AMINO ACID REQUIREMENTS OF ORAL ACIDOGENIC MICROORGANISMS ASSOCIATED WITH HUMAN DENTAL CARIES

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ALTHOUGH 100 or more different types of bacteria can be isolated from the oral cavity at any given time, relatively few have been implicated in the initiation of human dental caries. Those most frequently associated with this disease possess the ability to convert ingested carbohydrates into organic acids in amounts sufficient to degrade tooth structure. This common characteristic of diverse members of the dentobacterial plaque has led to the hypothesis that dental caries results from the combined action of oral acidogens rather than from the activity of any one species in particular.

There is a notable lack of uniformity in the acid-producing microbial composition of the dental plaque in different individuals and in the same individual at different times. On the basis of studies demonstrating species and strain specific differences in the vitamin B requirements of oral acidogenic flora, Dreizen and Spies[1] postulated that distinctions in nutritional essentials may contribute to the variability of the acidogenic component of the dentobacterial plaque. These studies have now been extended to establish the amino acid requirements of representative species of oral acidogenic microorganisms in pure and mixed culture. The assays were confined to the biologically active amino acids present in human saliva.[2-5]

EXPERIMENT I

Specific Amino Acid Requirements of Representative Species of Oral Acidogens.—

Method: Pure cultures of oral strains of *Lactobacillus acidophilus, Streptococcus salivarius, Staphylococcus albus,* and a glucose-fermenting Saccharomyces, isolated from patients with active dental caries, were carried as stabs in tomato juice agar. Inocula were prepared from twenty-four hour thioglycollate broth cultures of each organism in the manner described in detail in a previous publication.[6]

The specific amino acid requirements of pure cultures of each of the four test organisms and of a mixed culture of all four test organisms were determined in five series of sixty tubes. Ten milliliters of complete test medium or 10 ml. of each of nineteen different amino acid deficient test media were added to twenty sets of three tubes in each series. One drop of a saline suspension of a test organism was inoculated into two tubes in each set, the uninoculated tube serving as a control. The fifth series was inoculated with a mixture of all four test

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organisms. The chemical composition of the complete test medium is shown in Table I. With one exception, each of the nineteen different amino acid deficient media was identical in composition to that of the complete medium, except for the omission of a single amino acid. Both asparagine and aspartic acid were withheld in the aspartic acid assays. All tubes were incubated at 37° C. for seventy-two hours. Growth was measured in terms of the amount of 0.1 N NaOH required to restore the pH of the inoculated tubes to that of uninoculated control tubes. The titrations were made with the aid of a Beckman pH meter.

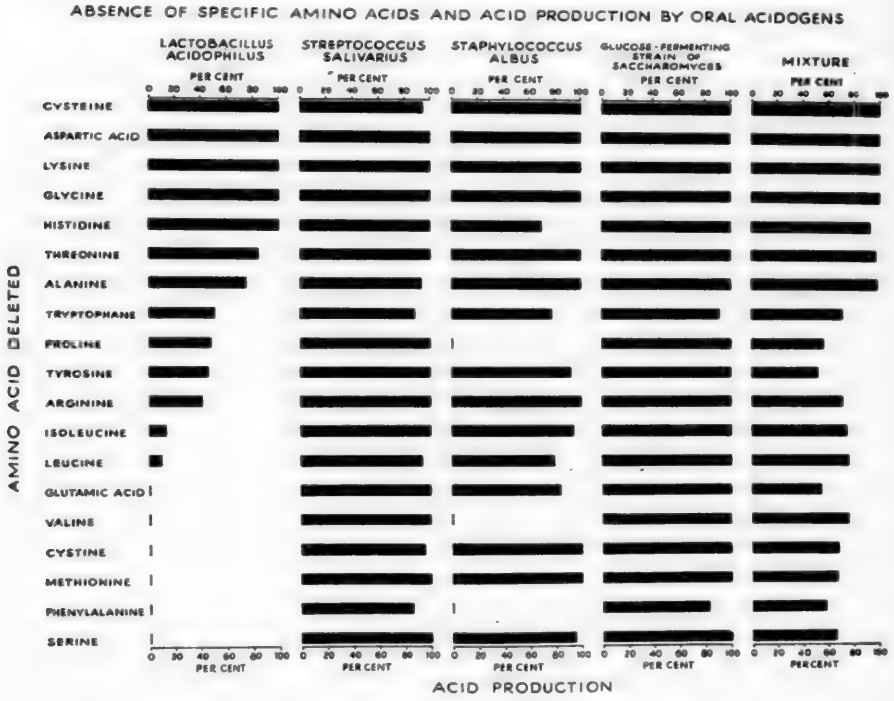

Fig. 1.

Observations: Fig. 1 shows the effect of withholding each of the nineteen amino acids from the chemically defined test medium on acid production by the four test organisms in pure and mixed culture. The quantities of 0.1 N acid formed in the complete medium during the seventy-two hour experimental period (100 per cent values) were: *Lactobacillus acidophilus,* 17.1 ml.; *Streptococcus salivarius,* 8.1 ml.; *Staphylococcus albus,* 3.5 ml.; glucose-fermenting Saccharomyces, 2.3 ml.; and mixed culture of the four test organisms, 13.7 ml. In contrast, growth of the *Lactobacillus acidophilus* was completely inhibited when glutamic acid, valine, cystine, methionine, phenylalanine, or serine was omitted from the complete medium. Acid production by this organism was significantly reduced in the tubes lacking either leucine, isoleucine, arginine, tyrosine, proline, or tryptophane. Growth of the *Staphylococcus albus* was completely inhibited

in the tubes devoid of either proline, valine, or phenylalanine. The test strains of *Streptococcus salivarius* and glucose-fermenting Saccharomyces were not affected by the omission of any one of the nineteen amino acids, provided the other eighteen were present in the test medium. Acid production by the mixed culture was significantly reduced in the tubes lacking the amino acids required by the test strain of *Lactobacillus acidophilus*. It approximated maximal values in the tubes lacking the amino acids not required by this organism.

TABLE I

COMPOSITION OF TEST MEDIUM

CONSTITUENT	QUANTITY	CONSTITUENT	QUANTITY
Asparagine	20 mg.	DL-Alanine	40 mg.
Adenine sulfate	1 mg.	L-Arginine	10 mg.
Guanine hydrochloride	1 mg.	L-Aspartic acid	10 mg.
Uracil	1 mg.	L-Cysteine	40 mg.
Thiamine hydrochloride	20 μg.	L-Cystine	40 mg.
Riboflavin	20 μg.	L-Glutamic acid	50 mg.
Nicotinic acid	20 μg.	L-Glycine	10 mg.
Biotin	0.4 μg.	DL-Histidine	20 mg.
Pyridoxine hydrochloride	40 μg.	DL-Isoleucine	50 mg.
p-Aminobenzoic acid	4 μg.	DL-Leucine	50 mg.
Folic acid	0.8 μg.	L-Lysine	20 mg.
Calcium pantothenate	20 μg.	DL-Methionine	40 mg.
Glucose	2 Gm.	DL-Phenylalanine	20 mg.
Sodium acetate	1.2 Gm.	L-Proline	10 mg.
Inorganic salts		DL-Serine	20 mg.
Salt solution A*	1 ml.	DL-Threonine	20 mg.
Salt solution B†	1 ml.	DL-Tryptophane	20 mg.
Distilled water to 100 ml.		L-Tyrosine	10 mg.
		DL-Valine	50 mg.

*Salt solution A	Amount per ml.
K_2HPO_4	100 mg.
KH_2PO_4	100 mg.
†Salt solution B	Amount per ml.
$MgSO_4 \cdot 7H_2O$	40 mg.
NaCl	2 mg.
$FeSO_4 \cdot 7H_2O$	2 mg.
$MnSO_4 \cdot 2H_2O$	2 mg.

EXPERIMENT II

The Chemical Classes of Amino Acids Required by Representative Species of Oral Acidogens.—

Method: To distinguish whether the amino acid requirements of pure and mixed cultures of the four test organisms were compound specific or class specific, acid production in the complete medium was compared with that in media of identical composition devoid of either monoaminomonocarboxylic acids, monoaminodicarboxylic acids, diaminomonocarboxylic acids, sulfur-containing amino acids, aromatic amino acids, or heterocyclic amino acids. The amino acids in each class are shown in Table II. The experimental procedure was essentially the same as that followed in Experiment I.

Observations: The classes of amino acids required by pure and mixed cultures of the four test organisms are shown in Table II. Each of the six classes was essential for the growth of the *Lactobacillus acidophilus*. Acid production by the *Streptococcus salivarius* was completely inhibited in the absence of the monoaminodicarboxylic acids or sulfur-containing amino acids, whereas, that by

the *Staphylococcus albus* was completely inhibited in the tubes lacking the heterocyclic amino acids. Neither organism required the diaminomonocarboxylic acids. In all other tubes seeded with pure cultures of either of these organisms, growth was submaximal. Acid production by the glucose-fermenting Saccharomyces was not affected by the lack of any single class of amino acids. In contrast, the growth of the mixed culture was significantly reduced in the absence of any of the six classes of amino acids.

TABLE II

EFFECT OF DELETION OF VARIOUS CLASSES OF AMINO ACIDS ON ACID PRODUCTION BY ORAL ACIDOGENIC MICROORGANISMS

GROUP OF AMINO ACIDS WITHHELD*	PER CENT OF ACID PRODUCTION				
	Lactobacillus acidophilus (17.1 ML. = 100%)	*Streptococcus salivarius* (8.1 ML. = 100%)	*Staphylococcus albus* (3.5 ML. = 100%)	SACCHAROMYCES SPECIES (2.3 ML. = 100%)	MIXED CULTURE (13.7 ML. = 100%)
Group I: Monoaminomonocarboxylic acids	0	78	37	100	53
Group II: Monoaminodicarboxylic acids	0	0	71	100	37
Group III: Diaminomonocarboxylic acids	0	100	100	100	59
Group IV: Sulfur-containing amino acids	0	0	66	100	34
Group V: Aromatic amino acids	0	51	80	100	38
Group VI: Heterocyclic amino acids	0	62	0	100	36

*Group I—Alanine, glycine, isoleucine, leucine, serine, threonine, valine.
Group II—Aspartic acid, glutamic acid.
Group III—Arginine, lysine.
Group IV—Cysteine, cystine, methionine.
Group V—Phenylalanine, tyrosine.
Group VI—Histidine, proline, tryptophane.

EXPERIMENT III

The Amino Acid Requirements of Mixed Cultures of Salivary Acidogens.—Method: The frequency of strain specific variations in amino acid requirements of the oral acidogens was determined by using cultures obtained from fifteen samples of paraffin-stimulated saliva collected from fifteen patients with a history and evidence of active dental caries. The generic identity of the acidogenic organisms in the mixed cultures was established by conventional bacteriologic technics. To prevent overgrowth, the mixed cultures were carried as tomato juice agar stabs, which were refrigerated until used for the preparation of inocula. The effect of the omission of each of the nineteen amino acids and of each of the six classes of amino acids on the growth of the fifteen mixed cultures was investigated in the manner described in Experiments I and II.

Observations: The generic distribution of the organisms contained in the fifteen mixed cultures recovered from caries-active saliva was: lactobacilli, 11;

streptococci, 15; staphylococci, 6; "yeast," 5; diphtheroids, 2; Neisseria, 2; and diplococci, 2. As shown in Table III, growth of these cultures was most inhibited (36 per cent) when glutamic acid was withheld from the test medium. Acid formation in the remainder of this series was reduced from 22 to 33 per cent of maximal when either tyrosine, tryptophane, arginine, isoleucine, leucine, valine, cystine, methionine, or phenylalanine was withheld. In cultures containing lactobacilli, the maximum inhibitory effect was obtained when, with the exception of serine, the amino acids required by the test strain of *Lactobacillus acidophilus* were omitted from the test medium.

Acid production by the fifteen mixed cultures of salivary acidogens was most inhibited in media devoid of either monoaminodicarboxylic acids or sulfur-con-

TABLE III

EFFECT OF DELETION OF AMINO ACIDS ON ACID PRODUCTION BY FIFTEEN MIXED CULTURES OF SALIVARY ACIDOGENIC ORGANISMS

AMINO ACID DELETED	MEAN ACID PRODUCTION (PER CENT)					
	GROUP I*	RANGE	GROUP IA†	RANGE	GROUP IB‡	RANGE
Glutamic acid	64	50-86	61	50-82	72	69-86
Leucine	67	18-93	59	18-93	90	88-92
Valine	69	30-93	63	30-93	88	77-92
Cystine	71	22-100	65	22-85	90	72-100
Methionine	71	23-100	65	23-85	88	80-100
Isoleucine	72	35-94	66	35-94	89	77-94
Phenylalanine	74	40-100	67	40-86	93	90-100
Arginine	75	63-85	75	63-85	76	71-80
Tyrosine	76	49-92	67	49-92	80	76-84
Tryptophane	78	60-90	78	60-90	78	69-88
Proline	91	47-100	93	47-100	89	74-100
Alanine	93	72-100	93	72-100	93	80-100
Serine	93	68-100	84	68-95	96	90-100
Histidine	96	85-100	97	89-100	93	85-100
Aspartic acid	97	90-100	97	90-100	98	94-100
Threonine	97	90-100	96	90-100	99	95-100
Cysteine	98	87-100	97	87-100	100	100-100
Glycine	98	88-100	99	91-100	97	88-100
Lysine	98	90-100	98	90-100	98	90-100

*Group I: Fifteen mixed cultures of salivary acidogens.
†Group IA: Eleven mixed cultures of salivary acidogens containing lactobacilli.
‡Group IB: Four mixed cultures of salivary acidogens not containing lactobacilli.

TABLE IV

EFFECT OF DELETION OF VARIOUS GROUPS OF AMINO ACIDS ON ACID PRODUCTION BY FIFTEEN MIXED CULTURES OF ORAL ACIDOGENIC MICROORGANISMS

CLASS OF AMINO ACIDS WITHHELD	MEAN ACID PRODUCTION (PER CENT)					
	GROUP I*	RANGE	GROUP IA†	RANGE	GROUP IB‡	RANGE
Monoaminomonocarboxylic acids	75	37-100	67	37-87	95	89-100
Monoaminodicarboxylic acids	15	0-55	13	0-51	18	0-55
Diaminomonocarboxylic acids	72	0-100	67	0-89	96	84-100
Sulfur-containing amino acids	20	0-66	21	0-66	18	0-55
Aromatic amino acids	84	73-100	82	73-100	91	80-100
Heterocyclic amino acids	71	46-100	70	46-87	74	50-100

*Group I: Fifteen mixed cultures of salivary acidogens.
†Group IA: Eleven mixed cultures of salivary acidogens containing lactobacilli.
‡Group IB: Four mixed cultures of salivary acidogens not containing lactobacilli.

taining amino acids (Table IV). In no instance did the quantity of acid produced in these tubes exceed 66 per cent of that formed in the control tube. Omission of the other classes of amino acids resulted in decreases of from 16 to 29 per cent of the amount of acid formed in the complete medium during the seventy-two hour experimental period. No distinction could be drawn between the classes of amino acids required by Lactobacillus-containing cultures and by Lactobacillus-free cultures.

DISCUSSION

The findings show a considerable variation in the amino acid requirements of oral acidogenic microorganisms. Six of the nineteen amino acids included in the chemically defined synthetic medium were absolute growth essentials for the test strain of *Lactobacillus acidophilus,* and three were required by the test strain of *Staphylococcus albus.* Conversely, none of the nineteen amino acids was essential for the test strains of *Streptococcus salivarius* or glucose-fermenting Saccharomyces, provided the other eighteen amino acids were available in the medium.

The oral acidogens exhibited strain specific as well as species specific differences in their amino acid requirements. While serine was an essential growth factor for the test strain of *Lactobacillus acidophilus,* it was not required by the strains contained in eleven mixed cultures. The wide range in the quantity of acid formed by mixed cultures containing lactobacilli and staphylococci in the absence of a specific amino acid or of a chemical class of amino acids (Tables III and IV) is also suggestive of strain specific differences in amino acid requirements.

Inhibition of oral lactobacilli and staphylococci in amino acid deficient media was much less pronounced in mixed culture than in pure culture. It would appear that some oral acidogens, particularly yeasts, can synthesize amino acids which act as growth essentials for lactobacilli and staphylococci. In contrast, the oral acidogenic bacteria appear to be limited in their ability to synthesize or transform amino acids into growth factors. These bacteria showed a greater specificity for particular classes of amino acids than for the individual amino acids, a finding indicative of a greater dependence upon the chemical nucleus characteristic of the class rather than upon the chemical configuration of the specific amino acid.

SUMMARY AND CONCLUSIONS

1. Species specific differences in amino acid requirements exist among the four oral acidogenic microorganisms most frequently associated with human dental caries. When grown in pure culture in a chemically defined synthetic medium containing all of the presently known nutritional essentials, the test strain of oral *Lactobacillus acidophilus* required glutamic acid, valine, serine, cystine, methionine, and phenylalanine. The test strain of *Staphylococcus albus* required proline, valine, and methionine. In contrast, growth of the oral strains of *Streptococcus salivarius,* glucose-fermenting Saccharomyces, and mixed culture of all four organisms was not affected by the deletion of any one of the nineteen amino acids studied, provided the other eighteen were included in the test medium.

2. Each chemical class of amino acids was essential for the three oral acid-producing bacteria in pure culture, whereas no single class was required by the glucose-fermenting Saccharomyces, provided the other five were present in the test medium. In contrast, acid production by the mixed culture of four test organisms was submaximal in the absence of any one of the six chemical classes of amino acids.

3. The amino acid requirements of the oral *Lactobacillus acidophilus* and *Staphylococcus albus* are strain specific as well as species specific.

4. The observations support the hypothesis that differences in nutritional requirements contribute to the variance in the acid-producing microbial spectrum of the dental plaque.

We wish to thank Dr. Karl Folkers of Merck and Company, Inc., for supplying the amino acids used in this study.

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EXPERIMENTAL DENTAL CARIES

II. Effect of Desalivation on Dental Caries and Castration and Desalivation on Fluorine Storage in the Rat

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MANY different workers have reported an increase in the caries experience of laboratory animals deprived of salivary gland tissue, with caries experience usually increasing concomitant with the amount of tissue removed by surgery or destroyed by chemical, radiologic, or physical technics. Kondo, Ichikawa, and Arai[1] have shown that extirpation of the parotid and submandibular glands of the rat resulted in a marked increase in the incidence of dental caries when compared with unoperated animals. Extirpation of the parotid glands alone resulted in a similar but less extensive increase in caries. These workers also noted that in both groups the mandibular teeth were affected to a greater degree than the teeth in the maxillary arch.

Cheyne[2] has reported the caries experience in rats which had various combinations of salivary glands surgically removed. His data indicated that the greatest incidence in caries occurred when both the parotid and submaxillary glands were removed, while the absence of the parotid gland itself, or the removal of the combined submaxillary and sublingual glands alone, did not approach the destructive effect of removal of the two sets of glands together.

Similarly, Hukusima,[3] studying the effects of submaxillary gland and combined submaxillary-parotid gland extirpation in rats, found that both procedures resulted in an increased incidence of dental caries as compared with controls.

Cheyne[4] also reported that fluorine reduces the incidence of dental caries in desalivated rats, there being an approximately 80 per cent reduction in caries experience of desalivated animals receiving 3 mg. of fluorine per day in the water supply as compared to desalivated rats not receiving added fluorine.

Gilda and Keyes[5] pointed out marked failure of weight gain in desalivated hamsters, as well as an increase in caries experience of significant proportions in both the female and the male animals. Similarly, Dale[6] reported an increase in caries in rats which had been desalivated, while also pointing out that in the two groups of desalivated animals used in his experiment there was a marked failure to gain weight as compared to a similar unoperated group. This weight difference was attributed in part to a lowered water consumption in the desalivated groups. Shaw and Weisberger[7] also indicated that desalivated cotton rats weighed considerably less in comparison to similar unoperated

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animals, and that a significantly greater number of carious lesions was found in the desalivated animals than in similar unoperated littermates maintained under otherwise identical conditions. Urban,[8] studying the effect of extirpation and x-ray irradiation of the salivary glands in rats, reported that xerostomia followed both procedures. These animals showed a higher caries incidence than controls, with the effects of extirpation being more marked than the effects of irradiation.

Klapper[9] reported that the hamster dentition was also affected by salivary dysfunction, for severe caries was found in the molar teeth of hamsters after desalivation. The caries pattern appeared to be reversed in the desalivated animals, for more caries developed in the first molar than in the third molar, an observation just opposite to the findings when unoperated animals were studied. "The severity and pattern of the experimental caries is in sharp contrast to that observed in nondesalivated animals and points to the important protective role of saliva in the production of dental caries in the adult Syrian hamster." Recently, Sognnaes and Shaw,[10] using desalivated rhesus monkeys, have reported that there is a significant reduction of P^{32} uptake in certain portions of the intact enamel surface.

Thus, most workers are impressed with the protective nature of the saliva on the teeth, while others attribute many diversified phenomena to the saliva, including an immunologic caries activity, such as the work with the salivary-ammonium ion concentration,[11] a medium for transporting basic systemic cellular and hormonal elements, such as in a saliva test for prenatal sex determination,[12] and influence on the general character of the periodontal tissues. The relationship of the salivary glands themselves to the structure and function of other body organs, including the endocrine organs, has not been firmly established, however. Ogata[13] was one of the early workers to ascribe an endocrine function to the salivary glands. Katagiri and Higashijo[14] have reported variations in the structure of numerous organs following the administration of estrogenic hormone in desalivated and intact animals. Alterations in the growth and development of the long bones in desalivated animals as compared with controls have been reported by Takizawa and his collaborators,[15-18] Miyasaki, Kondo, and Ichikawa,[19] and Ichikawa, Kondo, and Arai.[20] It is interesting to note that homotransplants of salivary gland tissue into the bone marrow of dogs have been singularly unsuccessful, although Ota[21] has stated that the parotid gland ducts do persist under such conditions for a relatively long period of time.

The magnitude of the work accomplished to date, as well as the rate at which work is progressing today, stresses the importance of this general problem. The purpose of this paper is to report the progress of a study on the general significance of the endocrine glands in their relationship to dental caries as may be influenced through the salivary glands. The important relationship existing between fluorine and dental caries may play a major role in this problem, and due to the proposed relationship between at least one of the endocrine glands and fluorine metabolism, a critical study has been designed to investigate the effect of these glands on the metabolism of this element. For example, it has been pointed out that tyrosine is a precursor for thyroxin as well as for some of the

adrenal hormones, and fluorine metabolism has been suggested to be partially under thyroid control. Thus, a portion of this paper specifically pertains to the correlation between dental caries in control and desalivated rats, and the relationship of fluorine administered in the drinking water to the incidence of caries in either control or desalivated animals. Since previous work from these laboratories has indicated that definite histologic changes result in the salivary glands after castration or injection of sex hormones in immature rats,[22] and since it is felt that some intimate relationship exists between the salivary glands, the endocrine glands, and fluorine metabolism, a portion of this paper deals with the analysis for fluorine of the skeletal system in desalivated animals and in animals receiving an androgenic and estrogenic hormone.

EXPERIMENTAL

A total of 120 rats* were divided into eight groups according to sex and weight; 114 finished the experimental period. Each male and female group consisted of a series of animals having the submaxillary and sublingual glands completely removed by surgery, along with at least three-quarters of the parotid gland. Because of the extreme diffuseness of the parotid, complete removal was probably not attained in this initial study. Another series consisted of a desalivated group which received 25 μg of fluorine per milliliter as stannous fluoride in the drinking water. A third group was unoperated and served as a control, while another unoperated group received the same concentration of fluorine as the desalivated group. The animals were operated on between 30 and 36 days of age, at which time the fluoride supplements were initiated. All of the animals received the cariogenic diet[23] beginning at birth, and they were continued on this regime until each animal was 140 days old. At this age the animals were sacrificed, and the heads were removed for dental caries studies and examined as previously described.[24] At the time each animal was sacrificed, it was given a number, so that on subsequent examination the identity of the animal, or the group to which it belonged, was unknown to the examiner. The femurs were taken for fluorine determination, as previously described,[25] and the uterus, testes, adrenals, thyroid, pancreas, and long bone were fixed for histologic examination.

DATA AND DISCUSSION

The data pertaining to the incidence of caries in the molar teeth of the experimental animals are shown in Table I. It is noted that desalivation increases the caries experience, as has been reported previously, in both the male and female animals, but to a different degree. In the females, desalivation appears to be about twice as effective in increasing dental caries as in the males. The reason for this is not known at the present time, although it has been previously noted in the hamster that the two sexes react differently to many experimental procedures. The inclusion of 25 μg of fluorine per milliliter as stannous fluoride in the drinking water has about the same effect in both sexes in reducing dental caries experience, but in the desalivated females receiving

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fluorine, the increase in dental caries as compared to the control females is quite evident, while in the males little noticeable change in the caries pattern resulted when these two groups were compared. Fluorine apparently inhibits cavity formation to an effective degree in the desalivated animals, for in both sexes there is noted a reduction in the caries experience of the desalivated animals receiving fluorine when compared with the desalivates not given fluorides.

TABLE I

THE EFFECT OF DESALIVATION AND ADMINISTRATION OF FLUORINE IN DRINKING WATER ON DENTAL CARIES IN THE RAT

GROUP	NUMBER OF ANIMALS	TOTAL WEIGHT GAIN (GM.)	NUMBER OF LESIONS	MOLARS AFFECTED	EXTENT	SIP*	CARIES CHANGE (COMPARED TO CONTROL DESALIVATES) (%)	CARIES CHANGE (COMPARE TO UNOPERATED CONTROLS) (%)
				Males				
Control	15	179	6.4	4.4	1.32	8.45	–16	0.0
Desalivate	13	87	7.6	4.6	1.62	12.30	0.0	+31
Control + F	15	149	5.1	3.8	1.01	5.15	–33	–39
Desalivate + F	13	92	6.5	4.5	1.25	8.13	–14	–4
				Females				
Control	15	111	5.8	4.0	1.10	6.40	–30	0.0
Desalivate	14	71	8.3	5.0	1.94	16.10	0.0	+60
Control + F	15	98	4.0	3.5	1.00	4.00	–52	–37
Desalivate + F	14	75	6.9	4.5	1.37	9.43	–17	+32

*Severity incidence product.

Removal of the salivary glands in both sexes caused a marked decrease in weight gain, which is somewhat greater in the males than in the females (Figs. 1 and 2). It is not known at this time if this failure of the desalivated animals to gain normally is due to the lack of saliva, which thus may induce a decrease in food consumption, or to other unknown factors. A paired-feeding experiment is in progress to evaluate the effect of food ingestion on weight gain in desalivated animals.

Table II shows the fluoride content of femurs which were analyzed as representative samples of the skeletal system from each group of animals. The procedure for fluorine determination was identical to that reported previously.[25] The experimental procedure did not appear to interfere with calcification of the femurs, as indicated by the similarity of the percentage ash in all the groups. Even though approximately fifteen to twenty times as much fluorine was stored in the skeletal system of those animals receiving stannous fluoride, no change in the quantitative chemical aspect of calcification occurred, as indicated by the ratio of calcium to phosphorus found in the femurs. Some data from this laboratory have indicated that desalivation of an animal causes subsequent change in other tissues of the body, but apparently no interference with fluorine storage in the femurs results from this procedure, as judged by comparison of these animals with the controls receiving similar concentrations of fluorine.

In connection with the relationship between desalivation and the endocrine system, it has been noted that certain androgenic and estrogenic substances

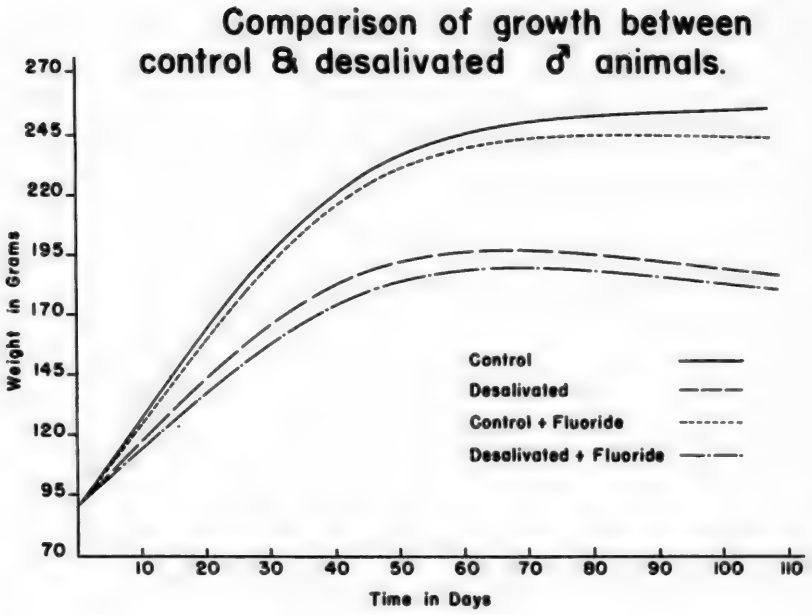
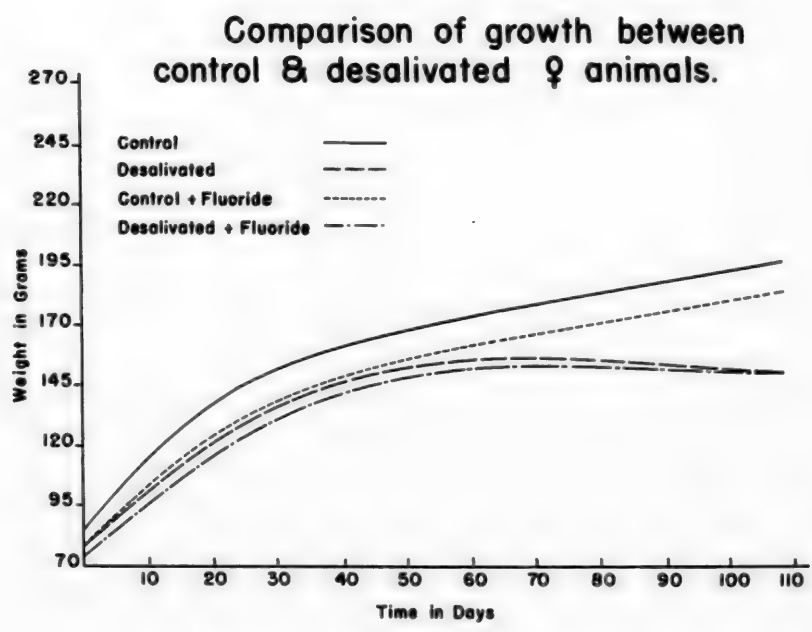

Comparison of growth between control & desalivated ♂ animals.
Weight in Grams
270
245
220
195
170
145
120
95
70
10
20
30
40
50
60
70
80
90
100
110
Time in Days
Control
Desalivated
Control + Fluoride
Desalivated + Fluoride

Fig. 1.


Comparison of growth between control & desalivated ♀ animals.
Weight in Grams
270
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40
50
60
70
80
90
100
110
Time in Days
Control
Desalivated
Control + Fluoride
Desalivated + Fluoride

Fig. 2.

TABLE II

THE COMPARISON OF FLUORINE RETENTION IN FEMURS OF DESALIVATED AND CONTROL RATS

GROUP	DESICCATED WEIGHT (GM.)	ASHED WEIGHT (GM.)	ASH (%)	FLUORINE CONCENTRATION (μG/GM.)	TOTAL FLUORINE (MG.)
		Males			
Control	0.480	0.273	57.2	381	0.105
Desalivate	0.430	0.245	57.0	323	0.077
Control + F	0.532	0.309	58.0	5,450	1.666
Desalivate + F	0.482	0.277	58.5	5,149	1.418
		Females			
Control	0.367	0.204	55.5	384	0.078
Desalivate	0.346	0.186	53.4	366	0.069
Control + F	0.415	0.226	54.8	4,533	1.035
Desalivate + F	0.400	0.217	54.1	4,976	1.073

TABLE III

A COMPARISON BETWEEN FLUORINE RETENTION IN CONTROL AND CASTRATED RATS RECEIVING DIETHYLSTILBESTROL OR TESTOSTERONE

GROUP	NUMBER OF LESIONS PER ANIMAL	SIP*	FLUORINE OF FEMURS					
			DESICCATED WEIGHT (GM.)	ASHED WEIGHT (GM.)	ASH (%)	F CONCENTRATION (μG/GM.)	TOTAL F (MG.)	POOLED SAMPLE OF F CONCENTRATION (μG/GM.)
				Males				
Control	8.0	14.50	0.519	0.272	52.3	261	0.0699	213
Castrate	6.5	8.25	0.502	0.242	48.0	173	0.0425	215
Control + testosterone	8.9	18.90	0.490	0.235	47.9	259	0.0611	252
Castrate + testosterone	7.4	12.62	0.473	0.262	55.4	281	0.0730	251
				Females				
Control	7.1	11.00	0.507	0.253	49.8	158	0.0398	290
Castrate	8.4	18.60	0.489	0.243	49.6	207	0.0459	346
Control + diethylstilbestrol	6.5	8.56	0.361	0.178	49.3	261	0.0468	275
Castrate + diethylstilbestrol	9.0	19.50	0.406	0.195	48.0	823	0.1610	743

*Severity incidence product.

apparently affect the storage of fluorine in the organism. Table III shows the incidence of dental caries after the administration of testosterone to the males and diethylstilbestrol to the females,[26] and the effect these hormones have on the storage of fluorine in the skeletal system of the rat.* No real differences in fluorine storage are noted in any of the groups studied except in the instance in which the synthetic female hormone was administered to the castrated females. It will be noted that the administration of this estrogenic substance increases the incidence of dental caries, as compared to a control female group,[26] as well as significantly increasing the storage of fluorine. There is no increase in fluorine storage in the control group which also received this same estrogenic substance, nor is any increase in storage of this element in the ovariectomized group alone noted. Furthermore, this same phenomenon did not occur in the male orchiec-

*We wish to thank Schering Corporation, Bloomfield, N. J., and Mattox and Moore, Indianapolis, Ind., for generously supplying the hormones used in these studies.

tomized animals receiving testosterone. It is difficult to explain these findings in terms of the commonly held fact that excessive administration or production of estrogenic hormones causes an increase in serum calcium and magnesium, or on the basis that estrogens themselves cause an increase in osteoblastic activity at the cartilage shaft junction, but not elsewhere. It was noted by one group of workers[27] that with the administration of estradiol there follows an increase in the density of the epiphyses and an increase in the concentration of the ash of the bone as a whole, but the total amount of bone is reduced because of decreased growth in length of the bones. It is noted in this work that the female controls receiving diethylstilbestrol have a somewhat smaller bone, but no interference in calcification is noted either in this group or in any other of the female series, as judged by a dramatic change in the percentage ash of any of the groups.

Table IV shows the relationship between the weight of the various groups of animals at the termination of the experiment and the weight of either the testes or uterus. In the males, the control animals' testes appear to be similar to the testes found in the other three groups, while in the females, the uterus is substantially larger in the controls than in any of the other three groups. If the data after repeated tests show the same findings, it must be concluded from these data that desalivation in the female causes marked atrophy of the uterine tissues. This interpretation might be correct were it not for the data on the total body weight of the desalivated females in comparison to the control females. Since these data indicate that there is an abnormal decrease in weight of the desalivated females, one is invited to believe that the weight decrease is due primarily to a lowered food intake. If this is correct, then the atrophy of the uterus in the desalivated females might be explained as being similar to the commonly observed atrophy of the uterus in laboratory animals resulting from food restriction. Nevertheless, this finding is being studied more extensively at the present time.

TABLE IV

A COMPARISON BETWEEN THE UTERUS AND TESTES IN DESALIVATED AND CONTROL RATS

GROUP	AVERAGE WEIGHT AT SACRIFICE (GM.)	AVERAGE WEIGHT OF ORGAN (MG.)	WEIGHT OF ORGAN PER 100 GM. OF BODY WEIGHT (MG.)
		Males	
Control	269	2,336	868
Desalivate	178	1,772	996
Control + F	239	2,291	959
Desalivate + F	182	1,847	1,015
		Females	
Control	195	435	223
Desalivate	151	200	132
Control + F	178	327	184
Desalivate + F	150	202	134

HISTOLOGIC EXAMINATION

Effect of Desalivation on Uterus.—Histologic examination confirmed the marked difference in size of the uteri between the control and desalivated groups

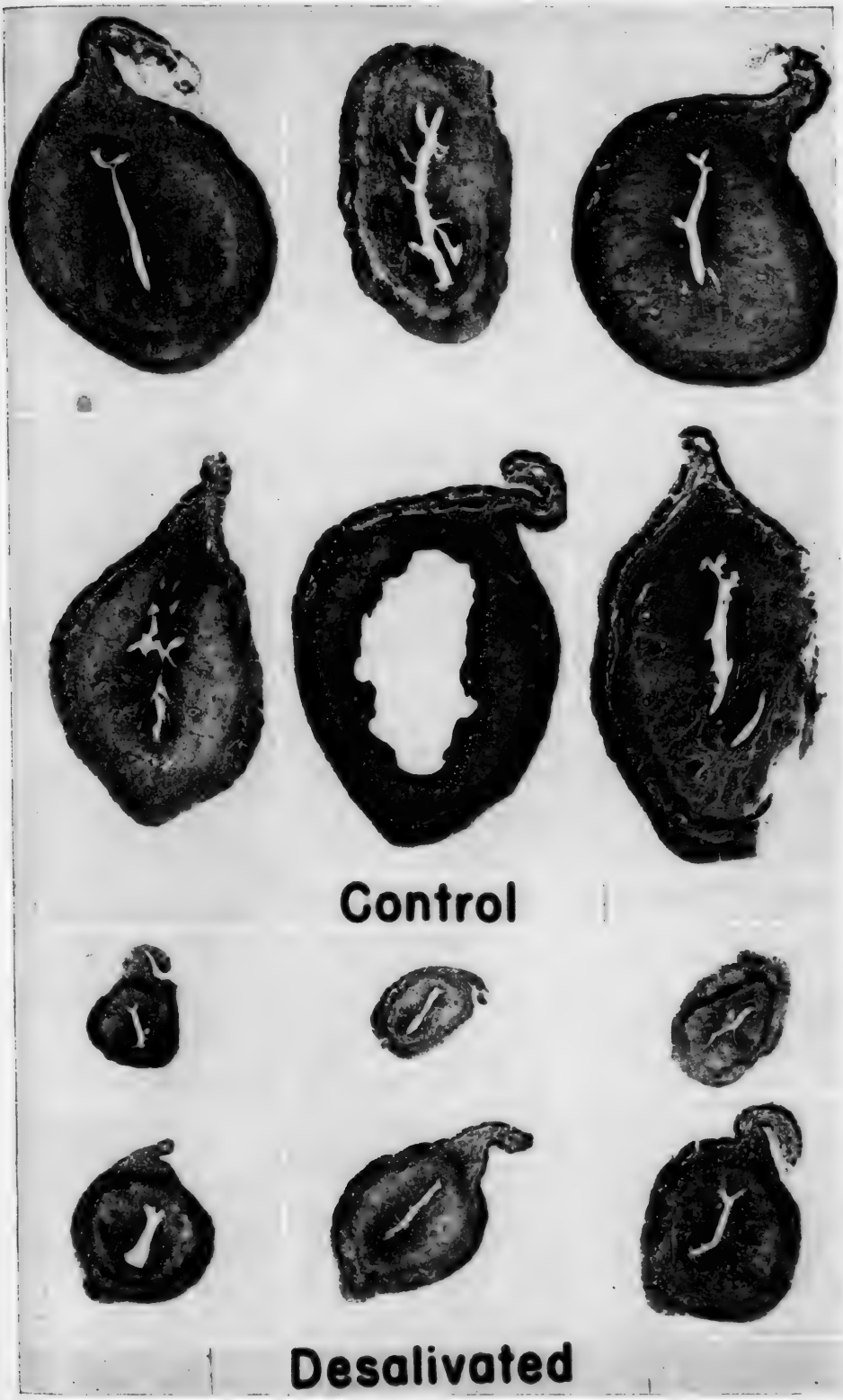

Fig. 3.—Photomicrographs of uteri of control and desalivated rats illustrating marked atrophy following salivary gland extirpation. All photomicrographs are reproduced at the same magnification.

of animals (Fig. 3). This difference was seen to be due to an apparent atrophy of the entire organ as a whole, and not to degeneration of any particular layer. The layers of the myometrium retained their normal relationship to each other in regard to comparative thickness. The desalivated animals, however, exhibited a generalized decrease in thickness of all three muscular layers. In general, there did not appear to be any significant decrease in the number of uterine glands present, although in the desalivated animals these glands appeared to be in a state of functional rest. Other changes noted were in the endometrium and the stages of the estrus cycle of the animals. In the control groups, as might be expected, the uteri were found in all stages of estrus and diestrus. The endometrium varied in type from flattened cuboidal to tall columnar epithelium, depending on the stage of estrus of the animal being examined. The degeneration of the epithelium and the penetration of leukocytes typical of the third and fourth stages of estrus were seen in a number of animals. The lumen of the uteri varied from a narrow slitlike opening to a widely distended opening characteristic of the first and second stages of estrus. In the desalivated group, however, all animals, with four possible exceptions, appeared to be in diestrus. In only one case was distention of the uterus noted, while vacuolization of the epithelial cells was not seen in any animal. In one case, the walls of the uterus appeared folding, while in two animals an extremely mild leukocytic infiltration was noted.

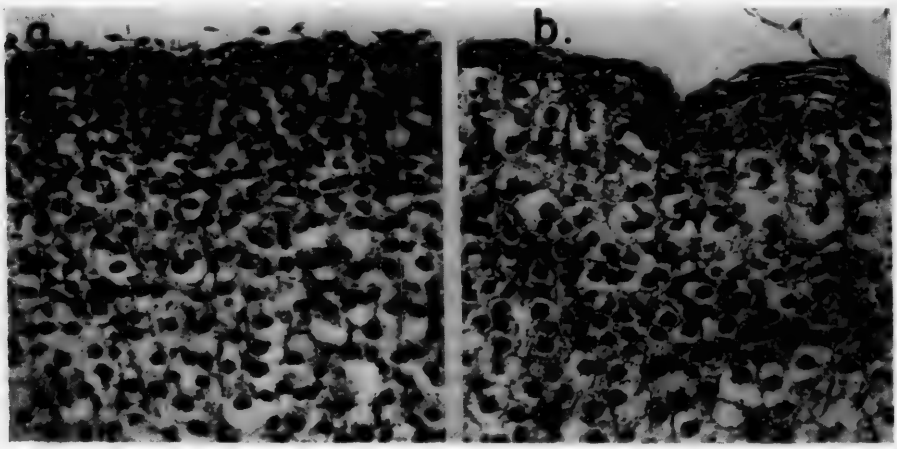

Fig. 4.—(*a*) Photomicrograph of zona glomerulosa of adrenal cortex from control female rat. (*b*) Similar area from adrenal cortex of desalivated female rat. The increase in lipoid-containing cells and dispersion of the band of spindle-shaped cells under capsule is evident.

Effect of Desalivation on Testis.—Histologic examination revealed no difference in testes of the various groups. The seminiferous tubules were comparable in all animals, and no abnormalities were noted in spermatogenesis. Neither were there any histologically detectable differences found between the interstitial cells of this organ in the various groups.

Effect of Desalivation on the Adrenal Gland.—Histologic examination revealed no consistent detectable difference between the adrenal glands of control

and desalivated male animals. It is of interest to note, however, that there was a marked change in the adrenal cortex of the female desalivated animals (Fig. 4, *a* and *b*). This change was so pronounced that the animals in nearly every case could be identified as control or experimental without recourse to records.

The variation noted in these desalivated animals was an alternation in the structure of the zona glomerulosa of the adrenal cortex. This outer zone in the control animals appeared compact, showed the component cells to contain a minimum of lipoid material, and exhibited numerous spindle-shaped cells arranged in a moderately wide band beneath the capsule. In the desalivated animals, the zona glomerulosa appeared to show an increase in thickness, the cells were swollen with lipoid material, and the band of spindle-shaped cells had become relatively inconspicuous. It was observed that this structural alteration, while also seen in the desalivated females receiving fluorine, was not as pronounced in the latter group.

The changes which were noted in the uterus of the desalivated females are very similar to those changes which have been observed in ovariectomized females. However, the alterations in the adrenal cortex of the desalivated females do not appear to be characteristic of those changes noted in gonadectomized females.

Effect of Desalivation on Thyroid, Pancreas, and Epiphyses of Long Bone.— Histologic examination revealed no detectable consistent differences between control and desalivated male and female animals in these tissues.

CONCLUSIONS

1. Removal of the submaxillary and sublingual glands, along with the majority of the parotid gland, in the rat results in an increase in dental caries. These effects of desalivation appear to be twice as destructive to the dentition of the female as to the male.

2. Fluorine, at a concentration of 25 μg per milliliter, effectively inhibits dental caries in desalivated rats to approximately the same degree in both sexes. No noticeable differences are evident in desalivated or control rats receiving the same diet in respect to the storage of fluorine in the femurs.

3. Desalivation appears to cause a generalized atrophy of the uterus of the rat as well as changes in the adrenal cortex of the female animal. The possibility that the changes observed in the uterus are due to a decreased food intake is not fully defined in this study.

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STUDY OF THE POSSIBLE RELATIONSHIP OF PERSONALITY VARIABLES TO DENTAL CAVITIES

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INTRODUCTION

FOR many years the hypothesis of a relationship between psychologic factors and bodily dysfunction has been discussed. A variety of experimental procedures has been applied, and certain pathologic entities have been accepted as having a psychologic basis.[4, 6-8, 11, 14, 19] Moreover, inferences have been made that oral pathologic conditions could have psychosomatic bases.[9, 15, 16] Most of these studies utilized chemical tests of the blood or saliva of institutionalized subjects,[10, 12, 17] or clinical observation.[9, 15, 16] The first attempt to establish a statistical correlation between dental disorders and psychologic factors was presented by Manhold and Manhold.[13] They administered the Bernreuter Personality Inventory to an unselected group of fifty people. Visual and roentgenographic examinations of the subjects' mouths were also conducted. From the latter examinations, a DMF (decayed, missing, filled) rating was established. Significant correlations were found between the resultant DMF rating and Bernreuter scores. These results indicated that further study might produce stable findings if a larger number of persons and varied personality measures were used. The purpose of the present study is to expand the former work in this manner.

METHODS

Guilford-Martin† and Bernreuter personality inventories,[1] and a DMF rating, were employed as variables for eight classes of Naval Aviation Cadets (266 subjects). The Guilford-Martin Personality Inventories consist of three questionnaries which purportedly measured thirteen traits of personality. The first questionnaire scores for Social introversion-extroversion, Thinking introversion-extroversion, Depression, Cycloid disposition, and Rhathymia. The second questionnaire scores for General activity, Ascendancy or leadership, Masculinity, Inferiority lack or self-confidence, and Nervous tension. The third questionnaire gives scores for traits which cover the temperamental area which "may roughly be designated by the term 'paranoid,' though only the extreme symptoms deserve that appellation borrowed from psychopathology."† This personality questionnaire, therefore, is purported to measure the "Psychotic Triad" by measuring traits of Objectivity, Agreeableness, and Cooperativeness. The Bernreuter Personality Inventory consists of 125 items which purport to measure six traits of

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†Guilford-Martin Personality Inventories, Sheridan Supply Co., Beverly Hills, Calif.

personality. Scores were obtained only on Neurotic Tendency and Introversion-Extroversion, the two traits which were formerly found to correlate with DMF.

To determine the DMF rating, the mouth of each subject was examined visually and roentgenographically. Cavities, fillings, and missing teeth were charted. Each tooth was then assigned a number which was computed on the basis of assigning one point to each surface of a tooth which contained a cavity or filling. Missing teeth were scored as three points in accordance with the Bodecker system[2, 3] and Marshall-Day's ACF (average caries frequency) rating.[5] The total was then obtained for each subject on the basis of a twenty-eight tooth dentition. This figure was the DMF rating. Third molars were not included in the computations. Decay around fillings was not counted unless it involved new surfaces.

RESULTS

For the total population, correlation coefficients were computed between DMF and the personality variables purportedly measured by the Guilford-Martin and Bernreuter inventories. Table I presents these results. Guilford-Martin Factors A, Ag, and Co were significantly related to DMF ratio at the 5 per cent level of confidence. These correlations were 0.12, –0.13, and –0.12, respectively. Subjects who rated themselves more ascendant (A), less agreeable (Ag), and less cooperative (Co) tended, on the average, to have more dental caries. Bernreuter traits, which had in the earlier study been found significantly correlated with DMF ratings, exhibited chance relationships.

TABLE I

CORRELATIONS BETWEEN PERSONALITY TRAIT SCORES AND DMF (DECAYED, MISSING, FILLED TEETH) RATIOS FOR THE TOTAL SAMPLE OF CADETS ($N = 266$)

GUILFORD-MARTIN TRAIT													BERNREUTER TRAIT	
G	A	M	I	N	S	T	D	C	R	O	Ag	Co	B_1 N	B_3 I
+0.06	0.12	–0.00	–0.03	+0.01	–0.09	–0.07	–0.01	+0.03	+0.09	–0.07	–0.13	–0.12	–0.03	–0.02

Extraneous factors operating on one or two classes of cadets might produce a significant correlation for the total sample. However, if personality variables are an important general factor in dental caries, they should operate the same for subsamples as they do for the total population investigated. A relationship similar to that found for the total population would therefore be expected to be shown by each of the eight classes.

With this in mind, correlations between DMF ratio and personality variables were computed separately for each class. Guilford-Martin traits A, O, Ag, and Co were selected for this special study since they had shown promise in the study of the total population. Bernreuter traits B_1 N and B_3 I were also considered, since in the study by Manhold and Manhold they had been found significant. They would also be useful as a comparison for correlations computed from subsamples where the total population correlation was zero.

Table II presents the correlations for each of the eight classes on the selected personality variables. Data were available for only nine subjects of

Class 42 and consequently this class should be disregarded in the interpretation of these results. A correlation of about 0.65 is required for a sample of this size to be significantly different from zero at the 5 per cent level of confidence. This value is so large that correlations computed on this size sample will only confuse the issue at hand. In interpreting Table II it should be recalled that there are two aspects to a correlation coefficient: (1) the direction of a relationship (either positive or negative) and (2) the magnitude of the relationship. For these classes, the direction of the relationship (the sign attached to the correlation coefficient) may be crucial to the interpretation of the results since the significance of the absolute size of the correlation coefficient is influenced greatly by the number of cases in the sample observed.

TABLE II

CORRELATIONS BETWEEN SELECTED PERSONALITY TRAIT SCORES AND DMF RATIOS FOR EACH OF EIGHT SAMPLES OF CADETS

CLASS	GUILFORD-MARTIN TRAIT					BERNREUTER TRAIT	
	N	A	O	Ag	Co	B_1 N	B_2 I
37	33	+0.32	+0.22	+0.02	+0.25	−0.06	−0.11
38	38	+0.14	−0.12	−0.20	−0.17	+0.03	+0.03
39	31	+0.02	−0.06	−0.10	−0.26	−0.30	−0.37
40	49	+0.23	−0.04	+0.01	−0.04	−0.01	+0.02
41	43	+0.00	−0.33	−0.30	−0.39	+0.05	+0.09
42	(9)*	(−0.59)*	(+0.02)*	(+0.42)*	(+0.25)*	(+0.11)*	(−0.04)*
43	28	+0.09	−0.06	−0.16	+0.22	−0.13	−0.17
45	35	+0.03	−0.08	−0.16	−0.32	−0.01	+0.04

*Correlations computed but disregarded in interpretation since the standard error for a sample of 9 is so large.

Guilford-Martin personality trait Ascendance (A) had been found for the total sample to be positively correlated with DMF ratings. For each of the seven subclasses (Table II) a positive relationship is likewise found. The probability that each of the seven subsamples will show by chance a positive correlation is less than the 1 per cent level of confidence. This probability is calculated from the relationship $(\frac{1}{2} + \frac{1}{2})^7$; the term needed is $(\frac{1}{2})^7$ or 1/128. The magnitude of these correlations is weak, as indicated by the fact that none of the correlations, in themselves, is significantly different from zero. These results suggest, therefore, that this trait would correlate positively on replication to new samples, but that large numbers of cases are required for significance since the relationship is a weak one.

A negative correlation had been found in the total sample between personality traits Objectivity (O), Agreeableness (Ag), and Cooperativeness (Co) and DMF rating. Negative correlations would thus be predicted for each of the subclasses. For personality trait O, six negative correlations and one positive are found (Table II). For personality traits Ag and Co, five negative and two positive correlations are observed. The likelihood that these results could occur by chance is 0.05 for trait O and 0.16 for traits Ag and Co. Thus, trait O (0.05 level) would most likely show a negative relationship when replication to new samples is tried. From the magnitude of the correlations observed, large samples of cases would be required since the relationship is a weak one.

The failure of personality traits Ag and Co to show significant negative correlations by the sign test applied to the subclasses may be more apparent than real. The failure is evidenced by the 0.16 probability value calculated from the sign test. However, for these traits, negative correlations for five of seven classes are in the expected direction. Furthermore, the absolute sizes of the correlation coefficients are generally larger than for the remaining personality traits investigated. The positive correlation coefficients for trait Ag are only 0.01 and 0.02, respectively. Thus, it would appear likely that trait Ag would also show a negative relationship to DMF ratings upon replication.

The findings for personality trait Co are not so easily generalized. The correlations noted in Table II for trait Co are relatively large for both positive and negative relationships found. The significant negative correlation found in the total sample for this trait seems to be a function of negative relationships for five subsamples counteracting relatively strong positive relationships for two other samples.

Thus, the separate analysis by class would indicate that the personality traits A, O, and Ag would be most likely to show the same relationships if replication to new samples of cadets were attempted. Although trait Co would also be predicted to show a significant negative correlation with DMF ratio, this prediction is held with less confidence than for the previous traits discussed.

The significance of the direction of the correlation may be determined from the fact that as trait scores increase, the trait is more pronounced. In like manner, the greater the DMF rating, the more decayed, missing, and filled teeth the subject possesses. Therefore, a positive correlation between Ascendancy and DMF means that as the purported leadership qualities of a subject increase, his dental caries also tend to increase. Likewise, a negative correlation between O, Ag, and Co scores and DMF may be interpreted to mean that the purportedly less objective, less agreeable, and less cooperative the subject, the greater his caries experience.

Bernreuter traits B_1 N and B_3 I appear to show correlations in the separate classes whose properties would be expected to arise from a population where the true correlation was zero. The fluctuations in the signs attached to the correlation coefficients, as well as their absolute size, indicate essentially chance relationships.

Item analyses were performed on the three Guilford-Martin inventories with the external criterion of DMF ratios. This was done in order to identify those individual personality items (as opposed to the personality scales) which might differentiate cadets with many, and those with few dental caries. These results failed to yield a meaningful pattern. The scales appeared to operate as a unit to produce the significant correlations noted.

DISCUSSION AND CONCLUSIONS

In this study, we are faced with the difficult task of correlating entities which are extremely controversial from many viewpoints. Dental cavity formation is a highly complex problem which is variously believed, for example, to

depend on diet, age, saliva, or the presence of specific bacteria. The measurement of cavity formation is an imperfect instrument, but the best available at present. In like manner, personality measurement is a maze of conflicting opinions and confused nomenclature. In addition, one of the main problems of psychosomatic medicine is encountered—the physiologic mechanisms by which psychologic factors could produce bodily dysfunction.

With reference to the first two problems, dental cavity and personality measurement, it may only be stated that deductions must be made from the best instruments available. The third problem, the physiologic mechanism by which personality variables may produce pathologic conditions, is in this case conjectural. Present-day theories of tooth decay, as stated, may be resolved into four main factors: (1) the effect of the ingestion of large quantities of carbohydrates; (2) change in saliva; (3) presence of *Lactobacillus acidophilus* or other acid-producing bacteria; and (4) dentobacterial plaque formation.

From psychologic literature, it is known that emotions can alter the pH of saliva and can vary the amount of food consumed by a person. Perhaps these factors can also influence other of these variables. Thus, it might be theorized that temperamental factors act as a catalyst for the action of bacteria or other media in the formation of dental caries.

Nevertheless, we do not purport to establish any explicit theories at this time. The approach to this study, rather, has been an attempt to establish statistically the fact that oral pathology and personality variables are interrelated. Consequently, the physiologic mechanisms have been disregarded to concentrate on the psychologic aspects of the problem.

With these considerations in mind, the results of this study offer some encouragement to the proponents of a psychosomatic basis for oral pathology. Significant correlations were established between DMF ratings and personality traits Objectivity, Agreeableness, and Cooperativeness. Furthermore, it would seem important that these traits lie in the same personality realm. These traits were designed by the test authors to tap the paranoid syndrome, a measure presumably of deeper strata of personality than those tapped by most of the other trait measures of the Guilford-Martin inventories.

On the other side of the ledger, the failure to replicate the findings for Bernreuter personality traits B_1 N and B_3 I reported by Manhold and Manhold earlier deserves some comment. One of the major differences which may account for this failure could be the relevant psychologic differences in two samples studied. In the study by Manhold and Manhold, Bernreuter scores for a population of unselected adults were correlated against DMF ratings. There is a distinct possibility that Naval Aviation Cadets represent a psychologically screened population as opposed to unselected adults with respect to the Bernreuter personality traits investigated. Men who apply for the Naval training program with obvious psychiatric problems would be rejected. This could result in restriction of range on the Bernreuter personality traits of neurotic tendency and introversion-extroversion. The failure to find significant correlations between B_1 N and B_3 I and DMF ratings in this study could be a result of this

restriction in range. As a check on the degree to which Naval Aviation Cadets have been selected, their means and standard deviations on the Bernreuter trait of neurotic tendency (B_1 N) can be compared to those for Bernreuter's normative group of male college students. The mean score on B_1 N for the Naval Cadet population of this study is –120.65 with a standard deviation of 72.48. The norm group of college men for this same trait has a mean of –57.3 with a standard deviation of 82.2. Thus, it is apparent that Naval Cadets rate themselves decidedly toward better "adjustment." than do college men generally. The difference is very extreme as shown by the fact that the two distributions have means about three-quarters of a standard deviation apart. The critical ratio for the difference is 11.6, significant far beyond the 1 per cent level of confidence. From this result, the failure to replicate earlier findings with respect to the Bernreuter test may be explicable.

The reasoning with respect to restriction of range should also hold for those Guilford-Martin traits most similar to the Bernreuter personality traits B_1 N and B_3 I. That is, the failure to find significant relationships for Guilford-Martin traits D, C, I, and N may also arise because the population of cadets has been selected with respect to these traits. Inspection of the mean score for traits D, C, I, and N and their comparison with the norm group of college students bears out this hypothesis, particularly for traits D and C. The mean scores for the cadet population are decidedly toward the "extroverted" direction for traits D and C and tend to be in this direction for traits I and N.

The possibilities of drawing definite conclusions at present are obviously few. However, the over-all findings of this investigation appear to point toward some type of relationship between personality variables and dental caries. The correlations found are relatively low, although statistically significant. Since a psychologically screened population was used in this study, the relationship may be somewhat attenuated by comparison with the population at large.

Assuming from the evidence in the literature that psychosomatic illnesses do exist,[4, 6, 7, 8, 11, 18, 19] we are of the opinion that, if temperamental factors are to be shown to correlate with measures of oral pathology, these factors should represent relatively more fundamental aspects of personality rather than those of a more superficial nature.

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THE EFFECT OF PROPYLTHIOURACIL ON THE DEVELOPMENT OF MOLAR TEETH OF RATS

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INTRODUCTION

IT HAS been shown that thiouracil and related compounds inhibit formation of thyroid hormone, producing much the same effect as thyroidectomy, but without the parathyroid disturbances often associated with surgery (Astwood, 1943; Astwood, Sullivan, Bissell, and Tyslowitz, 1943; and Hughes, 1944). It has also been shown that in the rat 6-n-propyl-2-thiouracil is approximately eleven times more potent than is thiouracil (Astwood, Bissell, and Hughes, 1945). Administration of propylthiouracil is thus an excellent means of studying the effects of thyroid hormone deficiency on the development of structures such as teeth or bone in which calcification plays such a prominent role.

Glickman and Pruzansky (1947) reported that propylthiouracil-induced hypothyroidism in albino rats caused retarded eruption of both incisor and molar teeth, as well as a reduction in endosteal and periosteal bone formation throughout the jaw. It was also stated that "appositional bone lines were more closely arranged, giving the bone a compressed appearance." The rats studied in this experiment received the drug through milk from mothers who were being fed 6-n-propyl-2-thiouracil at a concentration of 0.1 per cent in the chow.

English (1949), after administering thiouracil to a dog for a period of twenty-five weeks, at a dosage of 5 mg. per kilogram per day, reported hypoplasia in enamel, variation in size and shape of the condyle, and structural changes in the supporting bone. Haversian systems were smaller and irregularly arranged. Delay in tooth eruption following surgical thyroidectomy has been described in rabbits and swine (Kranz, 1914), in sheep (Todd and Wharton, 1934), and in rats (Ziskin, Salmon, and Applebaum, 1940b; Scow and Simpson, 1945). In sheep it was found that although eruption was delayed the order of eruption remained unchanged (Todd and Wharton, 1934).

The teeth were reported to be smaller after thyroidectomy in rabbits and swine (Kranz, 1914). Those formed after a similar operation in sheep were of normal size (Todd and Wharton, 1934). In rats which were thyroparathyroidectomized at birth or at 7 days, dentin and root development were retarded, but the size of the molar crowns remained unaffected (Ziskin, Salmon, and Applebaum, 1940b). It was found that after thyroidectomy in the monkey the amount of dentin deposited after operation was considerably reduced, and that this dentin was very poorly calcified (Ziskin and Applebaum, 1940a, 1941).

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In the present study, it was found that after establishing a state of hypothyroidism using 6-n-propyl-2-thiouracil, all of the processes associated with the formation and development of the teeth, that is, histodifferentiation, deposition, calcification, and eruption, were retarded, but to the same degree, so that the teeth developed in a normal manner but at a slower rate than normal.

After nine or ten days of drug administration there was apparent a considerable increase in density of the cancellous bone of the mandible of the hypothyroid animals.

MATERIALS AND METHODS

Seven litters of rats of the Long-Evans strain were reduced in number to six animals per litter. Each litter was divided into two groups of three, one of which served as an experimental group, the other, as a littermate control. An attempt was made to keep only the largest, healthiest animals in each litter for the experiment. The groups were, as closely as possible, equally proportioned as to sex.

Beginning the day after birth, each experimental animal received a daily subcutaneous injection of a 0.1 per cent solution of 6-n-propyl-2-thiouracil* in 0.9 per cent saline solution. The amount injected was as follows: 0.1 ml. from 1 to 10 days, 0.2 ml. from 10 to 20 days, and 0.4 ml. from 20 to 25 days. This dosage is the minimum required to produce the maximum effect with the least toxicity (Astwood, 1943; Hughes, 1944; Astwood, Bissell, and Hughes, 1945). Control animals received daily subcutaneous injections of equivalent amounts of 0.9 per cent saline solution.

Daily weight records were kept of the animals. Each litter group of control and experimental animals was weighed, and the mean weight recorded. A record was kept of the eruption time of the incisor teeth as measured by their appearance through the oral mucosa.

The rats were sacrificed in pairs, an experimental animal and its littermate control, at 5, 10, 15, 20, and 25 days of age. Two pairs were killed and prepared for study at each of these times.

The pituitary and thyroid glands were removed and fixed in Bouin's and Zenker's fixatives, respectively. These were subsequently embedded in paraffin, sectioned, and stained; the thyroids in hematoxylin and erythrosin, and the pituitaries in aldehyde fuchsin with light green (Romeis, 1940, Halmi, 1950, 1952).

The head of each animal was divided sagittally, and one half was fixed in Bouin's fixative, decalcified, and embedded in paraffin for section sagittally. The mandible of the other half was dissected out for grenz ray study. The maxilla of this half was fixed in Bouin's fixative, decalcified, and embedded in paraffin for section frontally.

Decalcification was carried out using the formic acid-sodium citrate method of Morse (1945). Heads were sectioned serially at 10 microns and stained with

*The 6-n-propyl-2-thiouracil used in this experiment was kindly donated by the Lederle Laboratories Division of the American Cyanamid Company, New York, N. Y.

hematoxylin and erythrosin. Grenz ray pictures were taken of each half mandible, using Eastman spectroscopic plates, type V-O.

OBSERVATIONS

General.—All of the animals which received propylthiouracil exhibited the characteristic retardation of growth associated with hypothyroidism. The weight curve of these animals and that of their controls are shown in Fig. 1.

The thyroid gland of each of the experimental rats appeared markedly hyperemic and hyperplastic with little colloid present. Delta cell vacuolation was demonstrated in the pituitary gland of animals which received the drug.

It was found that in those drug-treated animals which were not sacrificed until at least the age of 15 days, eruption of the lower incisor teeth was delayed from one to three days. In one animal the delay was one day, in four animals it was two days, and in one animal eruption was delayed three days.

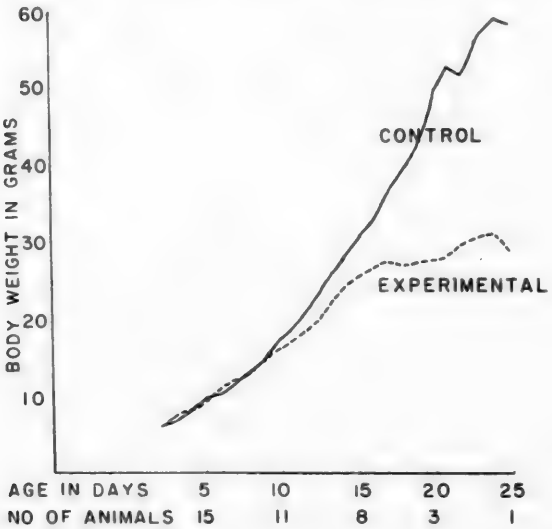


Fig. 1.—Weight curves of propylthiouracil-treated rats and their respective controls.

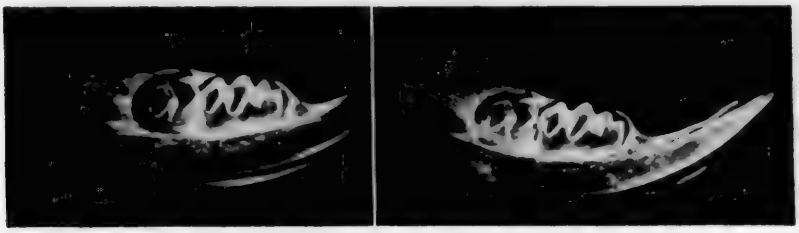
Grenz Ray.—Enlarged copies of the grenz ray negatives of the mandibles of two pairs of the animals are reproduced here. (Figs. 2 and 3.) A very small difference in the amount of tooth structure calcified was noted as early as 5 days of age, the control showing slightly more calcification than the experimental animal. This difference became more noticeable at later ages. At 15 days it was observed that the roots in the control first molar had begun to calcify, while in the experimental rat the same tooth had not yet reached this stage of development. The difference in amount of tooth structure calcified was most marked at 25 days.

By the age of 15 days, the retardation in eruption of the molar teeth in the treated group became noticeable in the grenz ray. At this time it was seen

that the control first molar had advanced farther out of the mandible than had its counterpart in the experimental animal. Retardation in eruption also became more marked at later ages and was most noticeable at 25 days (Fig. 3).

During the stage of development of each of the molar teeth when the enamel organ was growing larger, it was seen that the bone immediately surrounding the teeth in the mandible of the control animals was denser, and the dense area wider, than it was in the experimental animals (Fig. 2, second molars at 10 days).

At the same time, by 10 days, the bone of the condyle and body of the mandible appeared denser in the experimental animals. The trabeculae looked packed together, presenting, at 20 and 25 days, an appearance of almost solid bone, when compared to the normal lacy picture seen in the control mandibles.



A. *B.*

Fig. 2.—Reproductions of the grenz ray negatives of one half the mandible of rats 10 days old. *A*, Control. *B*, Propylthiouracil-treated.

A. *B.*

Fig. 3.—Reproductions of the grenz ray negatives of one half the mandible of rats 25 days old. *A*, Control. *B*, Propylthiouracil-treated.

A difference in size of the mandibles was apparent at 15 days, and this difference increased with age, being most noticeable at 25 days. In each instance the dimensions of the control exceeded those of the experimental animal (Fig. 3).

By 15 days it was apparent that the developing or odontogenic end of the incisor tooth was more distal in relation to the molars in the control mandibles than in the experimentals. This too was more noticeable at later ages, that is, if in the grenz ray negatives of specimens at 25 days (Fig. 3) a line perpendicular to the long axis of the body of the mandible was placed at the distal edge

of the third molar, it would, in the control, pass through the incisor tooth near its basal end. A similar landmark in the experimental mandible would pass distal to the end of the incisor.

Histologic.—

General: Histologically, in the propylthiouracil-treated rats there appeared to have been a gradual slowing down of all the processes involved in the formation of their teeth. Histodifferentiation, proliferation, deposition, and calcification all occurred in normal sequence, but at a slower rate than normal, compatible with the general retardation in metabolism associated with hypothyroidism.

The individual tissues associated with the teeth were not changed in any way that could be detected histologically as a result of administration of the drug. Differences between control and experimental specimens at the various ages were quantitive rather than qualitative; for example, at 15 days of age it was seen that more dentin had been deposited in the region of the cementoenamel junction of the first molar in the control animals than in the experimental ones, but there appeared to be no difference in quality (Fig. 4).

Enamel: At 5 days of age the enamel of the molar teeth of both control and experimental animals appeared the same, but by the age of 10 days it was apparent that more enamel matrix had been deposited, and calcification was further advanced in the teeth of the control rats than in those of the treated animals.

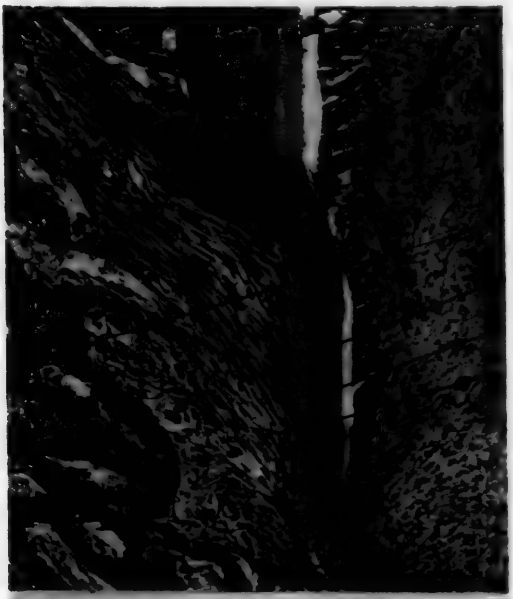
The enamel matrix appeared the same in both the control and experimental rats, and the difference in the amount deposited at each age studied from 10 days on was the only one which could be detected histologically. Calcification of the matrix followed deposition similarly in both groups of animals.

Dentin: As in the enamel, no structural differences could be detected in the dentin of the teeth of the control animals when compared with that of animals treated with the drug. At each of the ages studied, after five days more dentin had deposited at any given location in the control teeth than in the experimental teeth. In both specimens, however, calcification had followed deposition of the dentin matrix in a similar manner. The dentin-predentin border appeared smooth, and the width of the predentin zone was the same in both groups of animals (Fig. 4).

Cementum: Differences in the amount of cementum deposited also reflected the general retardation of growth in the experimental animals. By 15 days of age, cementum was visible on the roots of the first molar in the control animals, but none was present on the corresponding roots in the experimental rats. It was found in this latter location at 20 days, after sufficient root development had occurred.

Periodontal membrane: No change was observed in the periodontal membrane of the animals injected with propylthiouracil other than a delay in the transition of the loose irregular fibers of the dental sac to the directionally organized fibers of the periodontal membrane. This change from a loose connective tissue to an organized membrane was apparent around the roots of the

A.

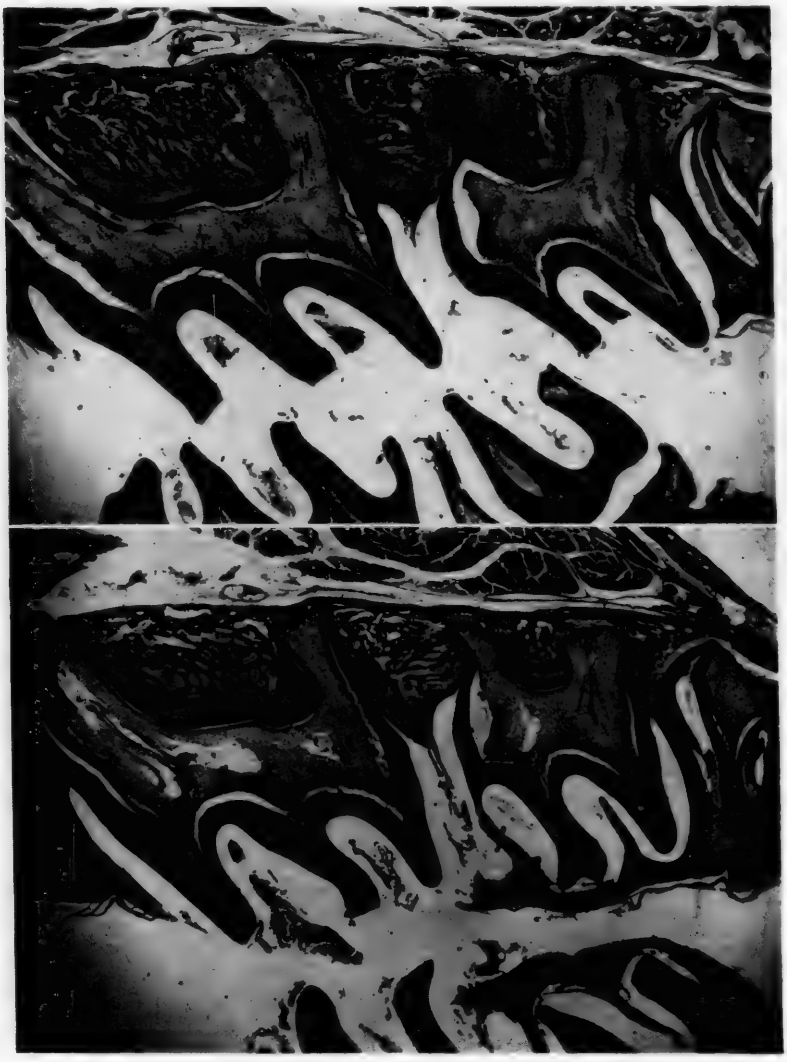


B.

Fig. 4.—Developing root of a rat maxillary first molar at 15 days. *A*, Control. *B*, Propylthiouracil-treated. Note that the organization of the periodontal membrane is less advanced in the experimental animal.

first molar in the control rats at about 15 days of age (Fig. 4), where fiber bundles may be seen running across the width of the membrane and attaching to both the tooth and the alveolar bone. Such organization was not apparent in the experimental animals at this time (Fig. 4). Organization of the periodontal

A.

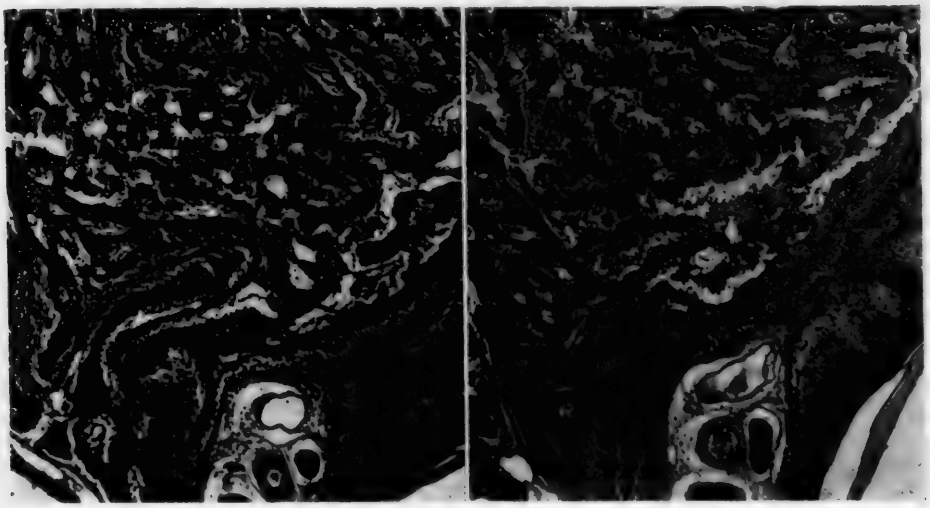


B.

Fig. 5.—Maxillary molars of 25-day-old rats. *A*, Control. *B*, Propylthiouracil-treated. Note the retarded eruption in the experimental animal.

membrane had occurred in the experimental animals by 20 days of age, after the root had developed sufficiently, cementum had deposited on the root, and the tooth had advanced farther toward the oral cavity.

Eruption: By 20 days of age the retardation in eruption of the molar teeth of the treated animals was apparent histologically. In the control animals at this age, the first molar had erupted into the oral cavity and the external enamel epithelium of the second molar had met and fused with the oral epithelium. The first molar of the hypothyroid animals had not erupted at 20 days of age, and the enamel organ of the second molar had not yet met the oral epithelium. At the age of 25 days, eruption of the first molar had occurred in the treated animals but was not as far advanced as in the control rats (Fig. 5). Cells of the epithelial attachment still had the anatomic characteristics of ameloblasts in the experimental first molar, while those in the same location around the control first molar were all squamous. Eruption of the second molar had occurred in the control but not in the experimental animals at 25 days.



A. *B.*

Fig. 6.—Frontal sections of 20-day-old rats' maxillae between the first and second molar teeth. *A*, Control. *B*, Propylthiouracil-treated. Note the thickened trabeculae in the experimental maxilla.

Enamel organ: The processes of histodifferentiation and proliferation were also retarded in the propylthiouracil-treated animals. No change other than retardation was observed. This was apparent from a study of the third molar. At 5 days of age the third molar in both groups of animals was in an early cap stage of development. From this age through to 25 days, the third molar in the experimental animals was behind its control counterpart in respect to rate of development. Differences were quantitative rather than qualitative.

Bone: At 10 days of age it could be seen that the bone trabeculae in the jaws of the propylthiouracil-treated rats appeared thicker than in the control jaws, with a concomitant reduction in marrow space. This difference was more readily apparent as the time of drug administration was prolonged up to 25 days. Fig. 6 shows photomicrographs of sections of the maxillae of two rats

at 20 days of age. These are frontal sections through the bone just distal to the first molar. Note the increased thickness of the trabeculae in the jaw of the propylthiouracil-treated animal (*B*) when compared with the control (*A*).

DISCUSSION

In this experiment, evidence or thyroid hormone insufficiency has been provided by, first, the retardation in growth demonstrated by the weight curves of the animals (Fig. 1) which is similar to that shown by Hughes (1944) after thiouracil administration from birth, and to that shown by Salmon (1938) after thyroparathyroidectomy at birth; and, second, the hyperplasia and hypertrophy with loss of colloid noted in sections of the thyroid gland of animals injected with propylthiouracil which is typical of the picture of thiouracil-induced hypothyroidism (Astwood, Sullivan, Bissell, and Tyslowitz, 1943). Vacuolation of delta cells of the pituitary gland has been shown to accompany hypothyroidism (Halmi, 1950).

The delay in incisor tooth eruption which was observed in this experiment has been commonly described following surgical thyroidectomy, and was reported by Glickman and Pruzansky (1947) after propylthiouracil administration.

The difference in size of the mandibles of the experimental and control groups was apparent in the grenz ray pictures at 10 days of age (Fig. 2), which is approximately the time that the weights diverge on the graph (Fig. 1). The more anterior position of the odontogenic end of the incisor teeth in the treated rats is probably a manifestation of lack of longitudinal growth in the mandible of these animals. Ziskin and Applebaum (1940) reported that after thyroparathyroidectomy at birth in the rat, there was a greater retardation in length of the mandible than there was in width. Silberberg and Silberberg (1943) found that in hypothyroid animals endochondral ossification was more retarded than was periosteal, and hence long bones appeared short and relatively thick. Since increase in length of the mandible depends chiefly on growth occurring in the endochondral center in the condyle, it follows that the greatest dimensional retardation in the mandible of a hypothyroid animal would be in length, rather than in width, which increases by periosteal ossification.

The grenz ray negatives illustrate the retardation of both development and eruption of the teeth of the treated animals. These pictures show only calcified portions of the teeth and jaws, but since calcification closely follows matrix deposition in both control and experimental rats, as was evident from the microscopic sections, the grenz ray pictures gave a fairly accurate indication of the state of development of the teeth at the different ages once the process of calcification had begun.

From the histologic study, it appeared that the teeth of the experimental animals developed in a similar manner to those of the controls except that they developed at a slower rate, similar to the retardation in appearance of ossification centers reported by Noback, Barnett, and Kupperman (1949) and by Weiss and Noback (1949) after thiouracil administration to rats.

No one developmental process was retarded more than another, and calcification appeared to follow matrix deposition normally in the treated rats. In no instance did it appear to be deficient. The predentin zone was in each case the same width as it was in the controls, and no zone of interglobular dentin could be found in any of the teeth of the animals.

This latter finding is not in agreement with the conclusion of Ziskin and Applebaum (1941) that the thyroid gland plays an important role in determining the quality of calcification of dentin as well as its rate of growth. After a discussion of the results of a series of three endocrine studies on the teeth and jaws, it is also reported in the same paper that thyroidectomy in monkeys produced more extreme effects than either hypophysectomy in monkeys (Ziskin and Applebaum, 1939) or thyroparathyroidectomy at birth or at 7 days in the rat (Ziskin, Salmon, and Applebaum, 1940b). In any endocrine study dealing with calcification of the teeth, one must take into account the status of the parathyroid hormone, which plays such an important role in tooth development (Erdheim, 1911; Toyofuku, 1911; Cohen, 1926; Jung and Skillen, 1929; Schour, Chandler, and Tweedy, 1937).

In their study on the effects of hypophysectomy on growing teeth in young rhesus monkeys, Ziskin and Applebaum (1939), using grenz ray pictures of ground sections of the teeth as a means of determining the state of calcification, found no interference with this process. The presence of a parathyrotropic hormone has never been demonstrated, and hence hypophysectomy would not be expected to influence directly the output of parathyroid hormone, which would continue to enter the blood stream at a rate commensurate with the metabolic rate of the animal; that is, there would be no relative parathyroid hormone deficiency in a hypophysectomized animal.

In the study of the effects of thyroparathyroidectomy at birth or at 7 days on the teeth and jaws of rats (Ziskin, Salmon, and Applebaum, 1940b), there was found a severe retardation in eruption time and in dentin and root development. No mention occurred in this paper concerning the state of tooth calcification, and no measurement was taken of this aspect of the teeth. It was nevertheless reported later (1941) that this study had led to the conclusion that calcification of teeth was not appreciably disturbed following thyroparathyroidectomy in rats. Salmon (1938), in her description of the operation on the rats used by Ziskin, Salmon, and Applebaum (1940b), stated that tetany frequently followed postoperative ether anesthesia in the rats, and that occasionally spontaneous tetany occurred, indicating that the animals were suffering from a low blood calcium, no doubt the result of a parathyroid hormone deficiency. It is not unlikely that if determination had been made of the state of calcification of the teeth of these rats, defects would have been found.

In the study of the effects of thyroidectomy and of thyroid stimulation on the growing permanent dentition of rhesus monkeys (Ziskin and Applebaum, 1940a, 1941), it was found that serious defects in calcification of the teeth followed thyroidectomy. This was apparent in grenz ray pictures of ground sections of the teeth and also histologically by an increase in the width of the

predentin zone. Injection of thyrotropic hormone into normal monkeys produced a slight increase in the width of the predentin zone, which was attributed to the increase in rate of dentin matrix formation. Grenz rays indicated that calcification was not disturbed.

In each of the thyroidectomized monkeys the parathyroid gland was removed at the time of operation, dissected free from thyroid tissue, and reimplanted into the sternohyoid muscle. No mention was made by Ziskin and Applebaum of any parathyroid disturbance. However, Jailer, Sperry, Engle, and Smelser (1944), describing in detail the results of the operation on the monkeys which were used by Ziskin (Applebaum, 1952), wrote as follows: "Four to five days after thyroidectomy the animals exhibited hyperirritability, tetanic convulsions, and even collapse. They were given parathyroid hormone subcutaneously and calcium gluconate intravenously. This was necessary for only a few days, after which these manifestations were no longer noted." Thus it appears that the monkeys were under an increasing parathyroid hormone deficiency for the four or five days following surgery until the blood calcium had become sufficiently low that tetanic seizures resulted, and under a decreasing deficiency for the following few days while treatment was given until the signs of tetany disappeared. That is, the monkeys appeared to be under a parathyroid hormone deficiency for at least one week, and may have been suffering from it throughout the full time of the experiment since disappearance of the signs of tetany did not necessarily indicate that the blood calcium level had returned to normal. It is suggested that the defects in calcification in the teeth of monkeys described by Ziskin and Applebaum (1941) were due to the parathyroid hormone deficiency rather than the thyroid hormone deficiency. In the present study, in which surgery was avoided and in which the parathyroid glands were not disturbed, no indication of hypocalcification was detected.

Increase in bone density associated with hypothyroidism has been reported by several authors. Thompson (1942) stated, "In hypothyroidism the urinary excretion of calcium is diminished and the bones sometimes show an increase in density." Silberberg and Silberberg (1943) described thickening of the long bones in cases of experimental hypothyroidism. Becks, Collins, Asling, Scow, Simpson, and Evans (1948) found that the bone of the mandibular head and neck was denser than normal in rats thyroidectomized at birth. The earliest age studied in this latter experiment was 56 days. Glickman and Pruzansky (1947), after propylthiouracil administration, found reduced endosteal and periosteal bone deposition, with the bone having a compressed appearance, and English (1949) reported that thiouracil administration in the dog resulted in bone changes. In the present study, increase in density of the cancellous bone in the experimental mandibles was apparent in the grenz ray and histologically as early as 10 days of age. The difference in bone density between control and experimental animals increased with age up to the age of 25 days. Bone trabeculae appeared more densely packed together in the treated animals and they appeared to have lost their orientation along the trajectories.

Increase in density of bone could be accounted for by the interference with resorption which accompanies experimental hypothyroidism (Silberberg and

Silberberg, 1943, 1949). Lack of orientation of trabeculae is possibly due to lack of function of the jaws of the treated animals.

SUMMARY

Rats were given daily subcutaneous injections of a 0.1 per cent solution of 6-n-propyl-2-thiouracil in 0.9 per cent saline solution beginning the day after birth. Littermate controls received 0.9 per cent saline solution by the same route and in equivalent amounts.

Animals were sacrificed in pairs, an experimental rat and its control at 5, 10, 15, 20, and 25 days of age.

Grenz ray pictures were made of one half the mandible of each specimen, and histologic sections were made of the developing molar teeth.

The following observations were recorded:

1. Cretinism occurred in each of the rats that had received the drug.
2. Retardation in development of the mandible was apparent in the grenz ray at 10 days of age.
3. At 15 days the odontogenic end of the mandibular incisor in each of the treated rats was farther forward in relation to the third molar than it was in the controls.
4. Retardation, but not cessation, of each of the processes associated with tooth development occurred.
5. By 10 days cancellous bone in the mandibles of the thiouracil-treated rats appeared denser than in the controls. This was apparent histologically and by grenz ray examination, and the difference increased with age to 25 days.

Grateful appreciation is hereby expressed to the members of the Department of Anatomy, College of Physicians and Surgeons, Columbia University, for their help in this work. Thanks are particularly due to Dr. E. Applebaum and Dr. W. M. Copenhaver for their kind advice and to Dr. H. Elftman who stained the pituitary sections.

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ORAL MANIFESTATIONS OF IONIZING RADIATION

II. Effect of 200 kv. X-ray on Rat Incisor Teeth When Administered Locally to the Head in the 1,500 r. Dose Range

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A SERIES of three experiments was performed to observe, among other things, the effects of a single dose of irradiation on growing incisor teeth of white rats during a relatively long period of time following irradiation (100 days). In the first experiment, the animals were divided into three groups, and different doses were given to each group. This was done to determine what dosage level might have an effect on the growth of the incisor teeth and still provide an adequate number of surviving animals for observation during the selected study period. An indication of the dosage range to be tried was obtainable from previous related studies on dental growth, made by Leist,[1] Burstone,[2] and Medak, Schour, and Klauber.[3] However, since we used different physical factors of radiation, with local head radiation only, using our own strain of rats, an actual trial was necessary. The second and third experiments were made to verify the changes observed in the 1,500 r. group of the pilot study.

METHOD AND MATERIALS

Two hundred ninety-eight weanling white rats of our own strain, 21 to 23 days old, were used in these experiments.* In the first experiment, 54 animals were divided into three groups of 18 animals each, and three separate doses of head irradiation were given, as follows: 500 r., 1,000 r., and 1,500 r. The group receiving 1,500 r. developed dental changes, as described under Results, and 12 of the 18 animals survived the whole experimental period (Table I). Therefore, 1,500 r. was selected as the dosage to be used in the second and third experiments. In the second experiment, 58 white weanling rats were given head irradiation, and 58 littermates, paired by sex and weight, were retained as controls. In the third experiment, 64 animals were irradiated (head only), and 64 littermates of the same sex and approximate weight were retained as controls. The animals used in the three experiments represent successive generations from the same breeding colony.

The equipment used in these experiments was a standard Picker commercial x-ray, the characteristics of which have been previously described.[4] The physical

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*The rats used have been maintained for six years, with periodic brother and sister mating to emphasize the uniformity of their characteristics. They have been found to have predictable caries susceptibility. They were originally from an Ausman-Mendle strain.

factors used were: 200 KVP, 20 Ma., filters ¼ mm. Cu and 1 mm. Al, half-value layer 0.82 mm. Cu, target skin distance 50 cm. The exposure time required for a dose of 1,500 r. under the physical conditions used was thirty-four minutes, five seconds (44 r. per minute). Animals were exposed in groups of 10. In order to restrain the movement of rats during irradiation and to be sure that only their heads would be exposed, they were placed in plastic boxes that had separate compartments for each animal. The heads of the animals were held in a fixed position by a tight-fitting, built-in neck stock, so that it was impossible for the head to be withdrawn from the field of exposure nor for the body to be thrust from under the protection of the lead shield.

TABLE I

MORTALITY AND GROSS OBSERVATIONS OF IRRADIATED RATS

	FIRST RADIATION SERIES (PILOT STUDY—500 TO 1,500 R.)			SECOND RADIATION SERIES (1,500 R.)	THIRD RADIATION SERIES (1,500 R.)
Total number of animals used	54			116	128
Number of animals irradiated	1,500 r. 18	1,000 r. 18	500 r. 18	58	64
Number of controls	–	–	–	58	64
Number of deaths 1st week among irradiated 2nd week animals Beyond 3rd week	5 1 –	2 – –	3 – –	7* 34 0	9 23 4
Number of animals which survived (sacrified at 100 ± 2 days)	10†	16	15	17	28
Gross observations of treated animals at 100 ± 2 days:					
Number showing eye opacity	2		–	14	25
Number with obvious loss of hair	12	10	–	16	20
Number with more than 2 external lower incisors	5	–	–	7	21
Number with missing or shortened upper incisors	3	–	–	3	4
Number of controls showing similar changes described previously	0	0	0	0	0

*Two died of asphyxiation at time of irradiation.

†Two animals with four external incisors were retained for breeding to observe possible effects on second generation. Offspring of these animals appeared to be normal.

After irradiation, the animals were maintained under constant conditions of temperature and humidity. One group of the animals was fed a cariogenic diet* because a secondary aim of this study was to note the effect of irradiation on caries. A careful record was kept of dietary intake and the weights of the animals. These factors and the caries results will be the subject of a later report. Periodic gross observations were made of the animals to observe changes in their hair, eyes, and general manifestations which might be caused by radiation.

In the third experiment, roentgenograms were made of the rats forty-three days after irradiation in order to observe the development of teeth and bone. The method of exposure was as follows: Animals were placed in plastic cylindrical tubes (3.5 or 4.5 cm. diameter, depending on size of animal), such as are used

*Corn caries diet: 20 pounds Quaker Yellow Hominy Grits, 10 pounds Klim, 1 pound alfalfa, ½ pound salt. First experiment, control diet: Purina Lab Chow. Second and third experiments, control diet: Rockland Rat Diet, pellet form.

in centrifugal machines. A small hole (1 to 1.5 cm.) was cut in the rounded end of the tube to permit the nose of the animal to protrude so it could breathe. A rubber stopper, with a 1 cm. hole to accommodate the rat's tail, was placed in the open end of the tube. Sometimes cotton packing was required in addition to the rubber stopper, in order to maintain smaller animals in a fixed position in the tube. The tube containing the animal was placed on the film holder under the x-ray machine, and the desired position was established by rotating the tube (Fig. 1). It was observed that rats seemed to be quite content in these close-fitting cylinders and actually appeared to go to sleep. This method has subsequently proved to be a satisfactory way of holding animals in a fixed position during longer periods of time, such as are required for local radiation procedures, thereby eliminating the disadvantages of using various anesthetics.

After 100 ± 2 days, the animals were sacrificed, and parts to be studied were immediately fixed in 10 per cent neutral formalin. Hard tissues were decalcified in formic citrate, imbedded in paraffin or celloidin, cut, and stained with hematoxylin and eosin.

Fig. 1.—Roentgenogram of unanesthetized rat in plastic tube illustrating position of rat in plastic tube. Note hole cut in rounded end of tube for nose to protrude and permit breathing. Hole in rubber stopper accommodates tail. Cotton packing is sometimes required behind smaller rats to hold them in end position, although several sizes of tubes are available. Rotation of tube permits any angulation to be obtained when roentgenograms are taken. Tube outline has been accentuated.

RESULTS

General Appearance.—Obvious gross effects (after 100 days) of 1,500 r. of x-ray administered locally to the heads of white rats were diminished body size, loss of hair in the exposed region, severe damage to the eyes resulting in opacity and blindness, and alteration of the incisor teeth (Fig. 2). During the second and third week following exposure many animals had matted hair about the mouth, lacrimated and salivated excessively, developed sores about the eyes, and tended to collect food particles and debris on their faces. Animals that succumbed were particularly disheveled in this way, and were rapidly losing weight.

Animals receiving 1,000 r. in the initial study were observed to lose some hair, but to a lesser degree than described in the case of animals receiving 1,500 r. Neither the 1,000 r. nor the 500 r. group was observed to have dental changes 100 days following treatment.

Change in Hair.—The loss of hair in treated animals occurred two to four weeks following exposure and was obviously absent across the nose and on top

Fig. 2.—Photographs of control animals (top row) and irradiated animals 100 ± 2 days following exposure to 1,500 r. of x-ray. Control animals above are littermates of six of treated animals shown below and are same age. Note that some maxillary teeth are missing in treated animals, and those that are present are irregular in size and coloration. Mandibular incisor teeth also show irregularities in size and color, and in addition there are three or four teeth present, instead of two as in the control animals. Elongated teeth traumatize the cheeks and gingiva of opposing jaws.

of the head. Occasionally, the neck or the front feet showed hair loss also, depending on the animal's position or ability to get his feet through the halter. Lack of hair was apparent for seven or eight weeks following exposure in many animals, but gradually most survivors developed new hair.

Skin Lesions.—From three to five weeks following exposure, certain animals developed skin lesions in the areas denuded of hair. These lesions persisted from several days to two weeks.

Eye Changes.—Opaque changes in the eyes of the rats were first noticed at about six weeks following exposure. Twenty animals developed this opacity in both eyes, and 21 on one side only. The opacity increased with age, and in no instance did it disappear. Animals with opacity in both eyes were unable to see.

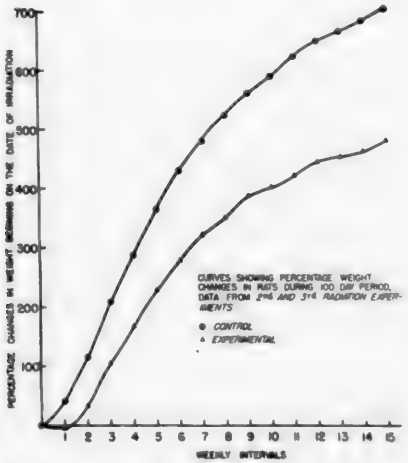

PERCENTAGE CHANGES IN WEIGHT BEGINNING ON THE DATE OF IRRADIATION
700
600
500
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
WEEKLY INTERVALS
CURVES SHOWING PERCENTAGE WEIGHT CHANGES IN RATS DURING 100 DAY PERIOD, DATA FROM 2ND AND 3RD RADIATION EXPERIMENTS
CONTROL
EXPERIMENTAL

Fig. 3.—Percentage weight change. During the first week, irradiated animals lost weight. They then began gaining weight at a rate slightly less than the control animals until the sixth or seventh week, when they started showing a more marked retardation in growth. By the fourteenth week, the irradiated animals were 30 per cent smaller (average) than the controls.

Weight Changes.—As shown in Fig. 3, the graphic presentation of weight changes indicated a loss during the first week, but the animals started to gain weight again during the second and third week. However, the irradiated animals never regained weight comparable to the controls. After seven weeks, the effect of irradiation on the treated animals was a marked reduction in percentage increase of weight. Many animals were in an emaciated condition after seven to ten weeks, and had dull and unkempt hair, excessive moisture about the eyes, nose, and mouth, and gradual appearance of malocclusion of incisor teeth.

Dental Changes.—

Mandibular: The characteristic changes in the incisor teeth were different in each jaw. In their mandibles, many of the animals (Table I) had what appeared to be additional incisor teeth, so that instead of the usual two, there were three or four teeth present. In these animals, only two teeth would be firmly fixed, the others being loosely attached to the bone. Inequalities in length

were frequent, and rarely was there normal occlusion between the upper and lower incisors. The loosened lower teeth were more often laterally disposed, were longer, and had the normal yellow-orange color. The firm teeth were often rounded incisally and were pale or mottled in color. Several animals had their labial lips perforated by long incisors, and the gingival tissues of some were inflamed or bleeding.

The *maxillary* incisor teeth did not exhibit as striking changes as the mandibular teeth in th 100-day-old animals. The usual alteration was an absence of one incisor or the occurrence of one or two short incisors. Usually when there was a short incisor, it was rounded incisally (instead of being chisel-shaped) and it was pale or mottled. The reason for the rounded tooth-ends and alteration in color, as will be seen from the roentgenographic and histologic studies, is that the teeth so involved represent a second growth from the tooth germ, following a complete stoppage of growth after irradiation. The original tooth is gradually extruded and may be lost at some time preceding the 100-day period. As a consequence of the second growth from the tooth germ, a new tooth follows along in the path of the first one. Whereas in the mandible the second growth was frequently lateral to the first formed portion of the tooth, in the maxilla the second growth followed in the same channel as the first, and forced out the original tooth. The maxillary incisor teeth which were rounded were those which were too short to be in occlusion with lower teeth and therefore unaffected by incisal wear.

There was a similar pathologic effect demonstrated in the incisor teeth of irradiated animals regardless of the diet, and likewise among the control animals there were no differences seen in the incisor teeth between the dietary groups.

Roentgenographic Manifestations.—In the third experiment, roentgenograms were made forty-three days after irradiation of the animals. At that time, sufficient changes had taken place in the developing incisor teeth so that the roentgenograms of the treated animals could steadily be differentiated from those of the control animals (Fig. 4). As a rule, the change in the maxillary incisor teeth could be detected more easily than in the mandible because of the definite space between the first and second segments of each maxillary tooth. In the mandible, the overlapping of the first formed segment of the tooth with the portion of the tooth which formed after exposure to 1,500 r. made it more difficult to discern the actual break between the two segments. At the forty-three day period, the second growth of the average maxillary tooth had formed an arc of more than three-quarters the usual length of a complete tooth. In the mandible, only about two-thirds of the tooth was represented by new growth.

Histologic Findings.—Sagittal sections through the maxillae and mandibles of rats 100 days following exposure to 1,500 r. showed that there was a temporary halting in tooth formation following exposure. In the maxillae (Fig. 5), there was usually a connecting strand of abnormal dentin toward the outer arc of the tooth. The dentin which was formed immediately following radiation was architecturally disarranged to the extent that no paralleling of dentinal

tubules existed. There were frequent void spaces and inclusions of cellular masses or indiscriminate matter. The space between the two tooth segments, or where the maxillary tooth was thinned, was filled with loose fibrous connective tissue containing vacuoles and islands of osteoid material. There were evidences also of enamel hypoplasia, not only at the site of the primary division of the tooth into two segments, but in the apical region as well (Fig. 6). At various spots, the ameloblasts were missing or altered beyond recognition as ameloblasts, and here one would find an invagination into the enamel of a mass of osteoid material, cellular clumps, and fragmentary material. It appears that even after 100 days there was not complete recovery of the odontogenic elements. There was an irregular junction between the dentin and enamel, and both the odontoblasts and ameloblasts were arranged in an irregular fashion.

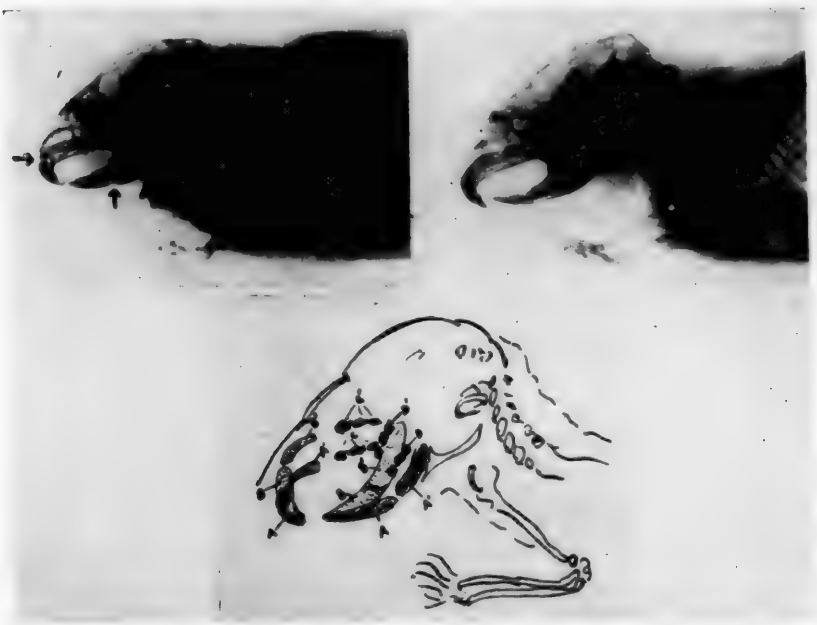


Fig. 4.—Roentgenograms of treated (*A*) and untreated (*B*) rats, both females, from same litter, and diagram (*C*) of treated animal, showing various segments of incisor teeth.

***A* and *B* were made forty-three days after treated animals received 1,500 r. of x-ray. Roentgenogram of treated animal (*A*) shows radiolucent zone in incisor teeth at arrows, indicating a halting of tooth formation during this period. Diagram shows foreshortened left incisor teeth *A* and *A'*, and elongated right incisors *B* and *B'*. The *A* and *B* portions of the incisor teeth were already present at the time of irradiation, whereas the *A'* and *B'* portions developed following irradiation, although for a period of time immediately after exposure, no tooth structure was formed. Molar teeth: *C*, *C'*, *D*, and *D'*. Note that treated animal (*A*) is smaller than control (*B*); comparative weights were 166 and 208 grams, respectively; weights at 21 days were 40 and 35 grams, respectively.**

The mandibular incisors of all animals which were treated showed a complete break between pre-exposure and postexposure tooth segments. In most instances, there was a lateral or superior-lateral overlapping between the two segments (Figs. 5, *C* and 7). It was observed that often there was a very wide pulp cavity in the pre-exposure segment of the mandibular incisors, indicating

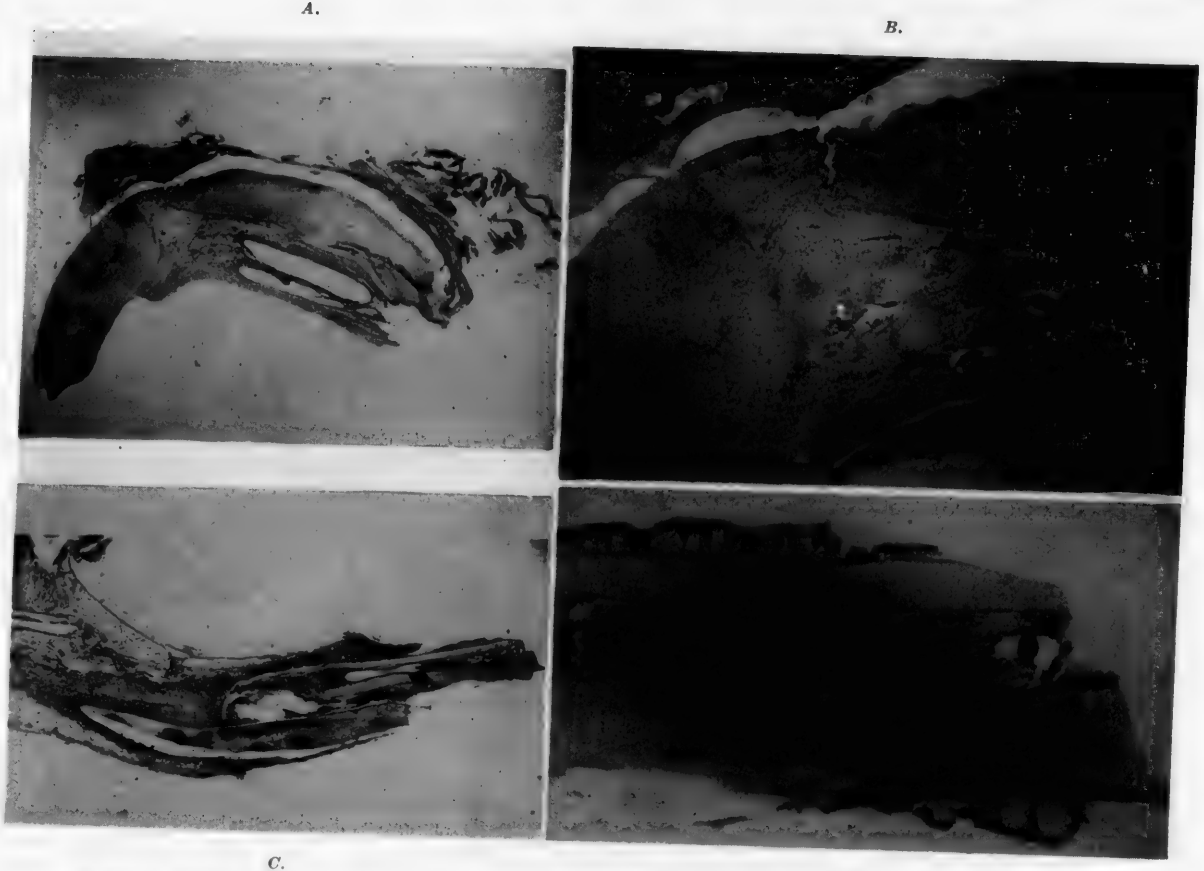
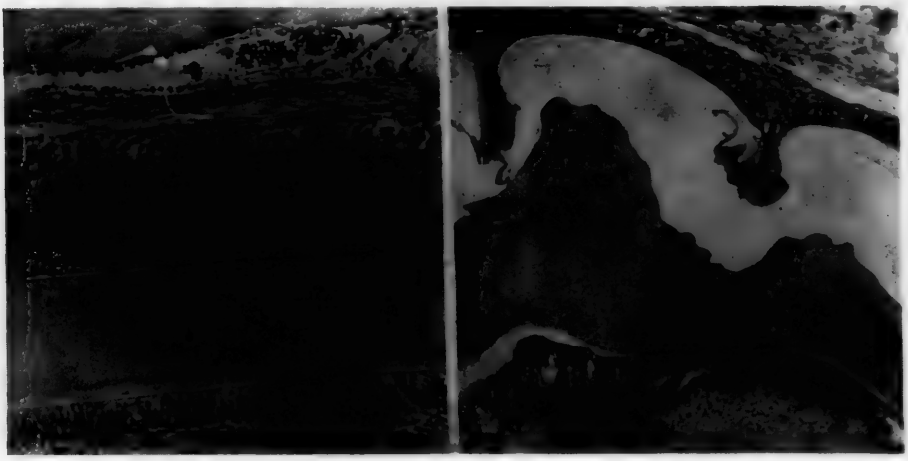


Fig. 5.—*A*, Sagittal section of maxilla of irradiated rat. (Original magnification ×6, reduced ⅙.) *B*, Enlargement of center portion of picture (*A*). (Original magnification ×20, reduced ⅙.) Note that maxillary incisor tooth is divided into segments, one external and one within the bony structure. There is a narrow bridge of poorly formed tooth structure between segments, indicating that amelogenesis was severely interfered with but not entirely stopped in this instance. The segment of tooth that was formed following irradiation is about two-thirds the normal thickness and contains some poorly formed dentin, indicating incomplete recovery of the tooth germ. *C*, Sagittal section of mandible of irradiated rat. (Original magnification ×6, reduced ⅙.) *D*, Enlargement of external end of *C*. (Original magnification ×40, reduced ⅙.) Note that there are two segments of incisor tooth in mandible, one above and external to the other. External segment has large pulp cavity because dentin ceased to be formed following irradiation. This portion of tooth continued to erupt and was worn down by mastication, exposing pulp chamber. At mesial end of this segment, abscesslike mass is seen, surrounded by leukocytes. Segment of tooth formed after irradiation is abnormal in architectural structure, indicating incomplete recovery of amelogenetic tissue

that the odontoblasts which had already formed a broad band of dentin at the time of exposure were destroyed by the irradiation. As a result of subsequent eruption of this segment and attrition by mastication, these teeth were sometimes worn until an open pulp chamber existed at the incisal end. This permitted entrance of debris and microorganisms. The area at the proximal end of the first segment and between the two segments of the mandibular incisor teeth was surrounded by polymorphonuclear leukocytes, with a central area of necrosis. An inflammatory reaction was usually evident well along the new-formed portion of the incisor tooth. Large masses of cementum-like material were present on the distal part of mandibular incisor teeth.



A. *B.*

Fig. 6.—Sagittal section through enamel, dentin, and their respective source cells, at the proximal end of the mandibular incisors, of (*A*) control and (*B*) irradiated rats. Note extreme regularity of ameloblasts (*1*), enamel (*2*), dentin (*3*), and odontoblasts (*4*), in the control animal (*B*). In irradiated animal the amelodentinal junction is wavy and the ameloblasts (*1*) and odontoblasts (*4*) are very irregular or missing in certain locations. A mass of irregularly formed calcified material projects into the enamel at the 2 o'clock position (*B*). These sections represent a growth segment that was formed between fifty and seventy-five days following irradiation. Considerable abnormality exists at this time, even though enamel and dentin are being formed.

In cross sections of mandibles of radiated animals (Fig. 7), the growth of a second tooth lateral to the original tooth segment was quite apparent. Here, again, it was observed that there were enlarged pulp chambers remaining in pre-exposure segments of mandibular incisor teeth due to premature stoppage in dentin formation. In other teeth, although the odontoblasts within the distal segment continued to function, markedly abnormal dentin resulted. In this dentin, there was usually a zone in which practically no dentin was present, crossed by radial strands which connected the preirradiation dentin to a second band of moderately dense dentinlike material. From this point to the broad pulp chamber only a lacework of structure was present. In some instances, there was actual resorption of tooth structure with a fibrous replacement. Frequent cysts were observed bordering the teeth. Polymorphonuclear leukocytes were present in pulp chambers of teeth that were worn down to the pulp cavity. The

Fig. 7.—*A* and *B*, Cross sections of the mandibles of two irradiated rats. (Original magnification ×20, reduced 1/6.) *C*, Enlargement of a portion of the primary tooth segment shown in picture (*B*). (Original magnification ×96, reduced 1/6.) Note that two segments of incisor tooth are present in *A* and *B*. In both cases, segment to left is from proximal end of portion of tooth which was present at the time of irradiation. There is poorly formed dentin in both of these structures and, in *B*, a large pulp chamber due to the early cessation of dentin formation. Note actual resorption of tooth structure in *A* at inferior border of primary tooth segment. Although tooth segments formed following irradiation approach normal (right of *A* and *B*), recovery of tooth-forming elements is not complete. In *C*, the pulp chamber is filled with leukocytes.

pulp chambers of the segments of teeth formed after irradiation were in the shape of small slits, as seen in normal animals. However, the dentin was not completely normal.

DISCUSSION

Tooth Eruption.—There have been occasional speculations in the literature concerning the location of the impetus for tooth eruption. Some evidence on this point seems to be available from these experiments. In the case of mandibular incisor tooth fragments which are no longer attached to the tooth germ because of the inhibition of odontogenic cells by irradiation, eruption seems to be extremely slow. In fact, one wonders whether extrusive forces, rather than eruption in the usual sense, are not the motivating factors. Such forces might be compared to the foreign body reaction which occurs in sequestration. This comparison in cases where an infectious process becomes established at the proximal end of the radiated tooth fragment is most apt. Extrusion of the primary fragment is slowed down to the extent that during the 100-day survival period a completely new mandibular tooth develops from newly differentiated cells of the survived tooth germ. The presence of the new tooth is evidence that were a normal eruption mechanism present, the first segment would long since have been lost. The eruption mechanism would thus seem to be closely related to the growth of the tooth from the tooth germ.

As demonstrated in the present study, there is a different sequence in the changes occurring in the maxillary incisor teeth following irradiation, as compared to the mandibular. In the former case, we find that the second growth from the tooth germ is anatomically directly behind the segment of tooth which was present at the time of irradiation. For this reason, a continued outward movement of the first segment seems to be necessary. In the mandible, there was a different sequence, as described. The reason for this difference between the upper and lower jaws is not readily evident, but several facts must be considered when theorizing as to possible reasons for the difference. The distance from tooth germ to the incisal end of the tooth in the case of the lower incisor is considerably greater than in the upper incisor, and eruption and growth are proportionally faster. Thus, leverage factors and eruptive forces are different, and in terms of distance traveled, more opportunity presents for deviation. It was also observed that some residual attachments usually remained between the two segments of maxillary incisor teeth, which may have provided a guide for the second segment.

Weight Changes.—The primary loss of weight of irradiated animals during the first week following irradiation is in keeping with the findings generally reported in radiation studies. The failure to recapture this weight loss during succeeding weeks and the marked leveling off in weight increase after about the seventh week suggest that secondary factors may be operative. The malformation of the incisor teeth, which begin making their external appearance at about this time, is a potential reason for weight loss, since they not only reduce masticatory efficiency, but subsequently serve as an actual obstacle to the intake of food. The inclusion of the pituitary gland in the area of irradiation is also a factor of obvious importance, both in general growth and in specific dental

changes. This factor may be eliminated as a variable in a future study by a more limited localization of irradiation.

SUMMARY

Three littermate groups of a well-controlled colony of white rats were used in a radiation study involving a single dose of localized head x-ray radiation to 21-day-old animals, with a maximum dose of 1,500 r. (200 KVP, 20 Ma., 0.82 mm. Cu half-value layer, to target skin distance 50 cm.). Striking changes were observed in the developing incisor teeth of irradiated animals sacrificed 100 days following treatment. Roentgenograms taken at forty-three days post-treatment revealed that all exposed animals already had a visible break in incisor tooth formation, located at the region which was forming at the time of exposure. The incisor teeth of sacrificed animals were separated into two segments: in the maxilla, the first segment was frequently lost at 100 days, leaving a stumplike tooth; in the mandible, the second-formed segment frequently grew lateral to the first, producing in effect an "extra" incisor tooth. As a result, many rats appeared to have four lower incisor teeth, whereas upper incisors were short or "missing." In histologic section, it was seen that extreme damage had been done to the tooth-forming elements which were physiologically active at the time of exposure, as evidenced by stoppage of tooth formation. The tooth-germ was also damaged, but to a lesser degree, as exhibited by the flaws in tooth formation during the 100-day period following irradiation. Although random odontogenic elements were completely obliterated, there appeared to be a general recovery of tooth-forming tissues.

We are greatly indebted to the staff of the Naval Medical Research Institute for generous support in this project. Specific thanks are extended to Russell Van Beloise and Clarence Miller for histologic technical aid, to C. R. Biles for radiologic technical assistance, and to C. E. Franer, J. S. McClelland, and E. E. Lawson for breeding, care, and observations of our animal colony.

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THE INDIRECT EFFECT OF X-RAY IRRADIATION ON THE DENTAL PULP OF THE DOG

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RADIUM and roentgen rays provoke edema,[34] increased permeability,[1, 24] and dilatation[5] of capillaries not only directly in exposed parts of the body, but also in adjacent areas or those remote from the site of irradiation. This indirect effect of irradiation depends on the development or increase of active substances, such as leukotaxins,[28, 36] histamine-like (so-called H substances)[4, 9, 10] and acetylcholine-like[12] bodies in the blood.

Since different stimuli, such as caries, physiologic and artificial abrasion affecting the outer surface of the tooth wall, induce reactions in pulp resulting in the formation of secondary or irregular dentin,[25] in this work an attempt has been made to produce these changes by means of x-ray irradiation of the dentin wall, while the pulp itself has been protected from exposure. In addition, an examination was also made to discover whether irradiation of the tooth crown causes alterations in the pulp of the root which would be protected from exposure. Until now, circulatory disturbances in pulp and alteration of odontoblasts with consequent formation of irregular dentin have been reported from experiments only with direct irradiation of teeth.[2, 3, 17, 22, 29, 35]

METHODS

Irradiation Technic.—For the experiment with a protected pulp of crowns, the four canines of a dog 1½ years of age were used. These teeth were selected because their crowns are sufficiently long, the dentin wall is fairly thick, the pulp chamber of the root is large (Fig. 4), and the physiologic regressive changes which might lead to misinterpretation of the results take place only in the very apical portion of the root (Fig. 8). For the protection of the pulp of the crowns, 1 mm. thick platinum shields were fitted into acrylic caps. The proper position of the platinum shields was checked by means of x-ray examination (Fig. 3). The crowns of the upper first and third incisors were exposed without pulp shields. For the protection of the roots of the eight previously mentioned exposed teeth and of the crowns and roots of the six non-exposed control teeth (lower incisors), lead shields were made (Fig. 1).

The radiation was obtained from a Douglas tube, using a tube voltage of 170 kv., a current of 7.1 Ma., and an additional filter of 0.5 Cu + 1 Al. The distance from the focus to the surface of the tooth was 50 cm. Such a long distance was advisable because the diameter of the focus spot was 11 mm. By

Preliminary results of these experiments were demonstrated in April, 1950, in a lecture at the Institute of Radiopathology of King Gustaf V's Jubileé Clinic, Stockholm, and were also reported in January, 1951, to the Medical Division of the Atomic Energy Commission, Washington, D. C.

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using a shorter distance, some radiation would have reached the pulp behind the platinum shield because of the geometric unsharpness. In order to avoid the exposure of the tooth through the opening of the lead shield, the central beam was directed tangentially to the plane of this opening. The field irradiated was less than 4 cm.^{2} Under the conditions described, 100 r. was obtained in four minutes, three seconds.

Fractionated irradiation was used for fifty-two to seventy-nine days. Since it was assumed that Tomes' fibers of dentin contain fine nerves[23] and that nerves belong to the most radioresistant tissue of the body,[14] high dosages of 8,900 to 19,500 r. were used for the teeth with platinum shields. The crowns of the teeth without platinum shields received smaller doses of 3,000 to 6,900 r. (Table I).

Prior to irradiation, the dog was narcotized with morphine-scopolamine, and trussed on a table. The head was fixed in specially constructed wooden clamps. The body and the nonirradiated part of the head were covered with lead rubber or shields. The survival period after the last irradiation was 206 days. Such a long period was chosen because, in teeth with limited growth, the development of suggested experimentally produced irregular dentin would be very slow.

Finally, the measurement of the amount of secondary x-rays which come from the irradiated crowns and reach the roots protected by shields was made.* For this purpose, canine in the mandible of another dog was used. The apical half of the root and the adjacent alveolar bone were removed. A small Sievert condenser dosimeter was placed in the cavity prepared in

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Fig. 1.—Lead shields for protection of jaws and roots of teeth 4+, 3+, and 4–, the crowns of which were irradiated.

Fig. 2.—The acrylic cap with fitted platinum shield placed over the crown of canine 4+. The crown of 3+ was irradiated without platinum shield.

Fig. 3.—Roentgenogram of crown of canine with acrylic cap showing that platinum shield completely covers the pulp. (Magnification ×4.)

Fig. 4.—Canine of dog in radiogram. Note the considerable thickness of dentin wall and the greater width of pulp chamber in the middle portion of the root.

Fig. 5.—Canine of dog in radiogram after grinding off enamel, cementum, and outer layer of dentin wall in order to reduce the duration of decalcification.

Fig. 6.—Measurement of secondary x-rays by means of Sievert condenser dosimeter. Ionization chamber (marked with number 31) is placed in the middle of the root, that is, in the space formerly occupied by the root.

Fig. 7.—Formation of thick collagenous coat around capillary in the same area of pulp as shown in Fig. 9. Hansen stain. (Magnification ×900.)

Fig. 8.—Root of canine 4+ irradiated with 19,500 r. The tooth wall became thin due to the grinding away of its outer layer. At the top and right of the picture, the dentin wall has been accidentally broken due to the excessive grinding off of the outer layers of the tooth wall. At coronal end of root, a large hemorrhage is seen. Note numerous distended blood vessels and fibrous metaplasia (physiologic) of pulp in the very apical portion of the root. Hematoxylin-eosin. (Magnification ×15.)

Fig. 9.—Pronounced edema of pulp due to irradiation of canine 4– with 16,450 r. In the center of the picture, there are three blood vessels with a radiant structure of collagenous coat shown in great magnification in Fig. 13. Cross section of root. Hansen stain.

Fig. 10.—Extensive hemorrhages, edema, and degeneration of pulp tissue due to irradiation with 9,200 r. of crown of canine –4. Longitudinal section. Hematoxylin-eosin. (Magnification ×50.)

Fig. 11.—Fibrinously thrombosed capillary in the pulp of crown of incisor 3+ irradiated with 6,500 r. Longitudinal section. Hematoxylin-eosin. (Magnification ×250.)

Fig. 12.—Thrombi in the area of apical foramen of canine +4 irradiated with 8,900 r. Cross section. Hematoxylin-eosin

Fig. 13.—Formation of collagenous coat of radiary structure around the arterioles of edematous pulp (see also Fig. 9). Coronal portion of root of canine 4– irradiated with 16,500 r. Hansen stain.

*For this measurement, I am obliged to K. J. Vikterlöf, M.Sc. (Institute of Radiophysics of King Gustaf V's Jubilee Clinic, Stockholm).

Fig. 1. Fig. 2. Fig. 3.

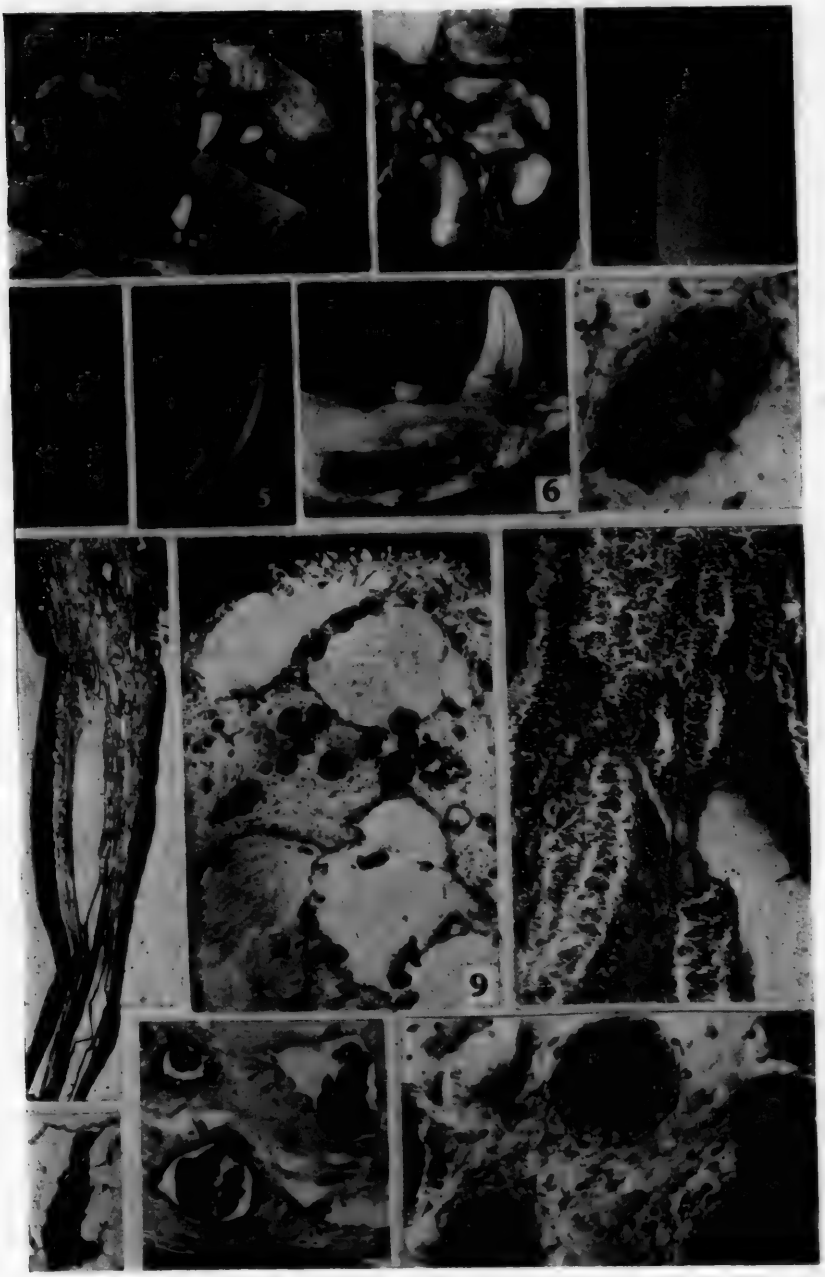

Fig. 4. Fig. 7.

Fig. 8. Fig. 10.

Fig. 11. Fig. 12. Fig. 13.

Figs. 1-13. *(For legend see opposite page.)*

TABLE I

THE FRACTIONATION SCHEME OF X-RAY IRRADIATION OF THE CROWNS OF THE TEETH OF THE DOG

NO. OF TEETH	NUMBER OF DAYS (DAILY SINGLE DOSES OF IRRADIATION IN R. UNITS)																						TOTAL DOSAGE IN R. UNITS
	1	2	4	8	12	16	20	23	27	30	34	37	44	47	50	53	57	65	68	72	75	79	
4 +	100	–	600	600	600	600	600	–	600	600	600	600	1,400	1,400	1,400	1,400	1,400	1,400	1,400	1,400	1,400	1,400	19,500
+ 4	–	400	–	400	400	400	–	400	400	400	400	600	600	600	600	600	600	600	600	600	600	600	8,900
4 –	50	–	800	800	–	800	800	–	800	800	800	800	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	16,450
– 4	–	200	200	200	200	–	200	200	200	200	200	200	800	800	800	800	800	800	800	800	800	800	9,200
3 +	–	–	–	–	–	–	–	–	300	300	300	300	300	600	600	600	600	600	600	600	600	600	6,900
+ 3	–	–	–	–	50	50	–	100	100	100	100	100	600	600	600	600	600	600	600	600	600	600	6,600
1 +	–	–	–	–	200	200	200	–	200	200	200	200	200	–	200	200	200	–	200	200	200	200	3,000
+ 1	–	–	–	–	200	200	200	–	200	200	200	200	200	–	200	200	200	–	200	200	200	200	3,000

this manner. The ionization chamber of the dosimeter was put in the space formerly occupied by the root (Fig. 6). The root and mandible were protected by the same shields used for the jaws of the experimental dog. The crown was irradiated from a Philips Metalix tube under the following constant conditions: 168 kv., 5 Ma., additional filter 0.5 Cu + 1 Al, half-value layer ~ 0.8 mm. Cu, irradiated field < 4 cm.^{2}, focal distance 40 cm. A dosage rate of 25.6 r. per minute was then obtained at the crown and 0.2 r. per minute at the root. The time of exposure was three to ten minutes.

Histologic Preparation.—The hard tissue wall of canines is fairly thick (Fig. 4), and it takes a long time to decalcify. The long time which is necessary for decalcification may account for one of the reasons for the formation of artifacts in the pulp. Since irradiation lesions, such as vacuoles and edema, can be interchanged with artificial vacuoles and edema which sometimes appear in tooth pulp as a result of histologic preparation,[16] the long period of decalcification was shortened considerably by means of grinding off the tooth wall. Immediately after extraction, the tooth was placed for about twenty to thirty minutes in a 10 per cent formalin solution. Then, all the enamel, the cementum, and the outer layers of dentin were ground off. In order to avoid the destruction of the pulp by the heat which develops during the grinding, the tooth was moistened with physiologic solution or 10 per cent formalin solution. Furthermore, in order to avoid grinding off too much of the dentin wall and thereby opening the pulp (Fig. 8), the grinding was checked with repeated examination by x-rays. My former experiences showed that when preparations of human teeth are made in this manner, there are fewer artifacts of the pulp. In this way, the decalcification of dog canines, which usually takes several weeks, could be brought about in a few days. A 5 per cent solution of trichloroacetic acid was used for the decalcification, and paraffin for the embedding. All the teeth were examined by means of longitudinal and cross sections. For the canines, serial sections were made. The slides were stained with hematoxylin and eosin, according to Bock (calcium stain), Montgomery, Hansen, or with Azan collagen stains.

RESULTS

The examination of crowns of canines showed heavy irradiation lesions of pulp in spite of the use of platinum shields. The same lesions, although of minor degree, were also observed in the roots in spite of their protection by lead shields. Findings of disturbances of circulation predominated. The distortion of blood vessels caused heavy hemorrhages and edema (Figs. 8, 9, and 10). In the lower left canine, the crown of which was accidentally broken by extraction, translucent exudate in a large amount poured from the open pulp. In the irradiated tooth, telangiectasis of veins, lymphatics, and capillaries revealed the richness of the circulatory network of the pulp (Fig. 8), which in normal teeth could be observed only by means of special methods.[19] Typical of the circulatory disturbances of the pulp was the appearance of hemorrhages and dilated blood vessels in the odontoblastic layer (Figs. 15 and 16).

A further peculiarity was the swelling of endothelial cells (Figs. 20, 21, and 25), which sometimes caused partial obliteration of the lumina of the vessels (Fig. 25). There was also a noticeable reduction and distortion of endothelium and of the muscles of media (Fig. 26). The destruction of the blood vessels caused metastatic thrombi and, thereby, also partial obstruction of the vessels in the area of apical foramen (Fig. 12).

Besides the swelling of endothelium, typical of irradiation lesions, the increase of collagenous matter was shown in the form of so-called "collagenous coat" of the blood vessels, chiefly the arteries. The collagen of the coat deposited showed fibrillar structure, or the pattern of fine network. This collagenous coat partially or totally surrounded the blood vessels (Figs. 7, 19, 20, 23, and 24). In a few cases, the collagenous coat of the arteries was of radiant structure (Fig. 13). The collagenous coat usually revealed none or only a few inclusions of cells.

Another type of increased formation of collagen appears in the pulp in areas between large bleeding or edematous spaces and the dentin wall. Here the pulp tissue is transformed into collagenous connective tissue rich in fibers and poor in cells (Fig. 22). In some compressed areas, the pulp tissue could be so severely damaged that instead of fibrous metaplasia, hyalin degeneration or necrosis took place (Figs. 10 and 17).

In spite of the high dosage of irradiation, the odontoblasts were generally less affected, and their distortion was local, depending on the influence of circulatory disturbances. When the distortion of odontoblasts was complete and sudden, as in the case of telangiectasia or bleeding in the odontoblastic

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Fig. 14.—Normal structure of odontoblasts in spite of irradiation with 6,600 r. Longitudinal section of crown of +3. Hematoxylin-eosin. (Magnification ×150.)

Fig. 15.—Local distortion of odontoblasts due to appearance of telangiectasia in their layer. Note narrowing of predentin in the area of lesion. Longitudinal section of root of canine –4 irradiated with 9,200 r.

Fig. 16.—Distortion of odontoblasts due to appearance of edema and hemorrhages in their layer. Note accumulation of extravasated red blood cells upon the edematous spaces. Longitudinal section of root of canine 4– irradiated with 16,450 r. Hematoxylin-eosin. (Magnification ×44.)

Fig. 17.—Degeneration of odontoblasts adjacent to area of degenerated pulp tissue. Note inclusions of odontoblasts in predentin and irregular canalization of dentin. Longitudinal section of crown of canine –4 irradiated with 9,200 r. Hematoxylin-eosin. (Magnification ×500.)

Fig. 18.—Disappearance of predentin, indicating gradual reduction of activity of degenerating odontoblasts in the course of development of edema in adjacent area of pulp tissue. Longitudinal section root of 3+ irradiated with 6,900 r. Hematoxylin-eosin. (Magnification ×300.)

Fig. 19.—Cross section of arteriole with formation of very thick collagenous coat. Note almost complete disappearance of endothelial cells. From the longitudinal section of root of canine –4 irradiated with 9,200 r. Hansen stain. (Magnification ×500.)

Fig. 20.—Cross section of blood vessel with swollen endothelium and collagenous coat in root of canine –4 irradiated with 9,200 r. Hansen stain. (Magnification ×600.)

Fig. 21.—Swollen endothelium cells of lymphatics in great magnification in root of canine 4+ irradiated with 19,500 r. Bock stain.

Fig. 22.—Fibrous metaplasia with rich development of collagen in pulp area compressed between dentin wall and large edematous area. Longitudinal section of crown of canine +4 irradiated with 8,900 r. Hansen stain. (Magnification ×44.)

Fig. 23.—Formation of connective tissue around blood vessels, indicating first stage in development of collagenous coat. Note diminution of cells in condensed tissue. Root of canine 4+ irradiated with 19,500 r. Hematoxylin-eosin. (Magnification ×150.)

Fig. 24.—Formation of thick collagenous coat around the thrombosed arteriole. Note fine network pattern and absence of cells in this coat. Root of canine –4 irradiated with 9,200 r. Hansen stain. (Magnification ×250.)

Fig. 25.—Narrowing of lumina of arteriole due to swelling of endothelium. Crown of the +3 irradiated with 6,600 r. Hematoxylin-eosin.

Fig. 26.—Degeneration of wall of arteriole. Note absence of endothelial cells. The longitudinal section of root of canine 4+ irradiated with 19,500 r. Bock stain.

Fig. 14. Fig. 15. Fig. 16. Fig. 17.

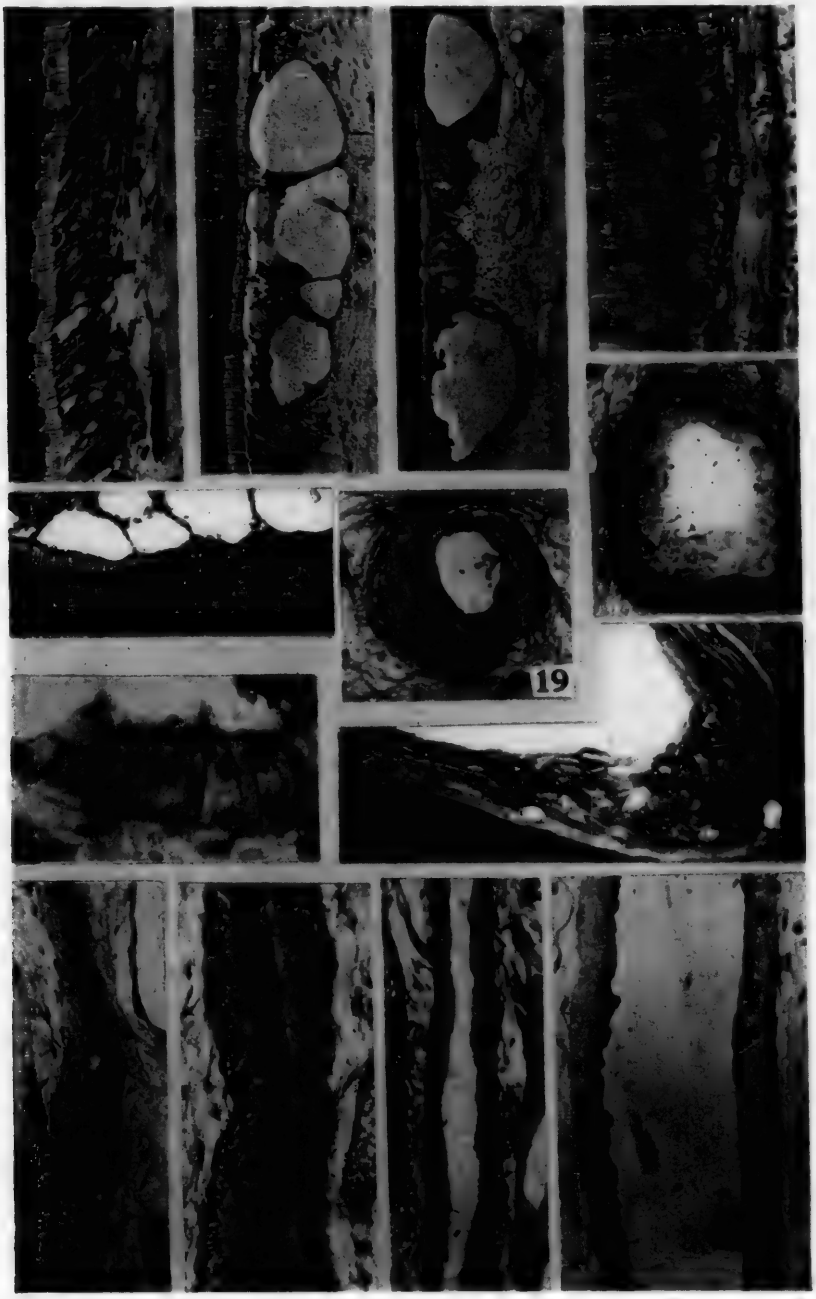

Fig. 18. Fig. 20.

Fig. 21. Fig. 22.

Fig. 23. Fig. 24. Fig. 25. Fig. 26.

Figs. 14-26. (*For legend see opposite page.*)

layer, no effect could be noted in the structure of dentin, with the exception of some narrowing of predentin (Figs. 15 and 16). If odontoblasts underwent a slowly increasing pressure for a long time from an adjacent edema or compressed pulp tissue, the gradual reduction of their function resulted both in the reduction of growth and in the formation of inferior dentin (Figs. 17 and 18). In the case where single odontoblasts stopped their activity while the neighboring cells were still capable of formative function, the former (that is, the single odontoblasts) became embedded in dentin formed by the neighboring cells (Fig. 17).

The very apical portion of the roots showed the usual course of regressive changes typical of normal teeth with limited growth. The fibrous metaplasia of pulp tissue was of the same appearance in them as in the nonirradiated (control) teeth. Bleedings were less pronounced, and edema was absent.

The examination of teeth (the upper third incisors) without platinum shields, which received smaller amounts of x-rays (6,600 r. and 6,900 r.) showed telangiectasia and hemorrhages of a somewhat lesser degree than in canines. The edema was not pronounced here. The roots of these teeth also showed telangiectasia and swelling of endothelium. The odontoblasts, if they were not destroyed by hemorrhages, were of normal appearance (Fig. 14). The pulps of the upper first incisors with the minimum dosage of x-rays (3,000 r.) showed only slight lesions in the form of some telangiectasia, which were seen in both the crown and the root, similarly to the teeth with a high irradiation dosage. Control teeth, with both crowns and roots protected, revealed a normal histologic structure.

DISCUSSION

The results of these experiments in which there was irradiation of the dentin wall only, the pulp being protected by platinum shields, do not allow any definite conclusions. It is difficult to say whether the circulatory disturbances observed in the pulp had been caused indirectly by means of ionization of Tomes' fibers in the dentin wall from irradiation, or whether the pulp was directly affected by x-rays. In the latter case, the reasons would be unsatisfactory elimination of geometric unsharpness of the central beam or secondary radiation from the calcium salts of dentin.

In the pulps of the roots, in spite of their protection with lead shields, the dilatation of vessels, edema, swelling of endothelium, and increased formation of collagen were of the appearance and degree which have been regarded by other investigators as typical of irradiation.[5-8, 11, 13, 20, 21, 26, 27, 30, 32, 33]

It might be expected that eventually secondary x-rays from the irradiated crown would affect the pulp of the root. By the measurements described (Fig. 6), it was found that the dose received by the root as secondary radiation was only 0.8 per cent of the dose received by the crown, and therefore such a small dose (24 to 140 r.) could not be of practical significance for the changes observed. The lesions in the protected pulp of the root may depend on the indirect effect of x-rays due to the formation of so-called H substances or leukotoxins in the irradiated pulp of the crown.

Previous investigators reached no uniform conclusions concerning the increase of collagen around the blood vessels (Figs. 7, 13, 19, 23, and 24). On the one hand, the deposition of collagen was regarded as a possible consequence of telangiectasis[30]; on the other hand, it was assumed that this collagen formation might be a secondary feature of inflammation of the surrounding connective tissue.[21] The material presented in this work shows that the collagenous coat developed chiefly in arteries and for the most part in those which showed only a slight dilatation or were not dilated at all. Generally, there were no inflammatory changes in the area where this collagenous coat was deposited. The appearance of the collagenous coat may be a result of a response of adventitia cells to the pressure from the edematous areas surrounding the corresponding vessels. It is noticeable that with its radiant structure the coat is suggestive of the appearance of predentin.

The same explanation may be given for the increased formation of collagen in the compressed area of the pulp between edematous areas and dentin (Fig. 22). Here, pulp tissue undergoes fibrous metaplasia of the same type as it did physiologically in the very apical portion of the root due to the narrowing of the pulp chamber, and is similar to "Presspulpa"[18] (compressed pulp).*

The swelling of endothelial cells of blood vessels (Figs. 20, 21, and 25) has been regarded as a typical feature of irradiation lesions.[5, 8, 11, 26, 28, 32] In our experiment, it was observed in blood vessels and lymphatics. After the examination of skin vessels, carotid, and aorta, one of the earlier investigators[27] concluded that, in some cases, the endothelial swelling might be an optical illusion produced through the loss of collagen and muscle of the vessel wall, making the endothelium appear relatively prominent. Since normally even the large blood vessels of the dental pulp scarcely show any condensation of fibrous tissue around them (which might serve to form the usual adventitial tunica), and since they only contain separate involuntary muscle fibers which represent media,[19, 31] the endothelial swelling observed in this experiment proves that this alteration really is a genuine irradiation lesion.

It was observed that developing odontoblasts are more radiosensitive than adult ones.[2, 22, 29] In a previous experiment of mine,[17] it was shown that mature odontoblasts, if they were not destroyed because of local circulatory disturbances, remained almost unaffected even after a dosage of 4,200 r. (fractionated irradiation by 143 kv.). The present work shows that the odontoblasts are capable of producing regular dentin in those crowns which received 6,900 r. (Fig. 14). In the canines with platinum shields, the circulatory changes were more pronounced than in the teeth mentioned previously, and it must be assumed that these teeth received more than 6,900 r. of x-rays. Since normal odontoblasts could be observed here also, it must be assumed that mature odontoblasts are among the most radioresistant cells of the body.

*Since this work was completed, Jolles and Koller have reported that during treatment of tumors by the sieve method, fibrosis was induced in regions protected from irradiation.

SUMMARY AND CONCLUSIONS

The indirect effect of x-rays on the pulp of crowns and roots was studied in eight teeth of a dog.

1. An attempt was made to produce changes in the pulp of the crown by means of irradiation of the dentin wall only, while the pulp itself was protected by platinum shields. The findings observed do not allow any definite conclusions to be made as to whether the resulting changes in the pulp depend on the effect of indirect or direct irradiation.

2. Owing to the indirect effect of irradiation, changes such as telangiectasis, hemorrhages, edema, increased formation of collagen around blood vessels in the form of collagenous coat (sometimes of radiant structure), fibrous metaplasia of pulp tissue, swelling of endothelium in blood vessels and lymphatics appeared in the pulp of roots in teeth where the crowns were exposed, but the roots were protected by means of lead shields.

3. Since normal odontoblasts were observed in spite of irradiation by doses of more than 6,900 r., it is assumed that mature odontoblasts belong to some of the most highly radioresistant cells of the body.

4. The reduction of the period of decalcification of teeth (during histologic preparation) by means of grinding off the outer layer of the tooth wall has been described.

I wish to express my gratitude to Associate Professor R. Thoraeus (Institute of Radiophysics of King Gustaf V's Jubilee Clinic, Stockholm) for his advice, help, and teaching, and to Professor B. Karlström (Medical Department of the Royal Veterinary College, Stockholm) for allowing me to perform this experiment in his department.

I am obliged to Mrs. G. Törngren for making the histologic preparations and to Mr. H. Kabe (Division of Cancer Research at King Gustaf V's Jubilee Clinic, Stockholm) for the photomicrographs.

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MEASUREMENT OF THE ELECTRIC CONDUCTIVITY OF DENTAL CEMENT

I. Method and Values for Dry Specimens

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ONE of the recognized problems associated with restorative dentistry is the thermal and electric conductivity of the various materials employed. Related to this problem, of course, is the presence of galvanic currents in the mouth due to cells produced from metallic restorations. The clinical significance of these currents is as yet unknown. The use of cement bases underneath gold and amalgam restorations has long been advocated to provide an insulating barrier against thermal and electric injury to the pulp. However, little study has been made to determine the actual insulating properties of these materials, and no reference to the electric conductivity of dental cements can be found in the literature. Therefore, the purpose of this project was to investigate the electric conductivity of materials which are used as cement bases for restorative work.

It was necessary to devise methods and apparatus for determining the electromotive forces, electric resistance, and electric currents associated with metallic dental fillings. Some early work concerning the electric aspects of dissimilar metals was carried out by Lain.[1] Schoonover and Souder[2] studied the corrosive action of galvanic currents in externally constructed cells, while Lain also has shown that dental cements which contain a high per cent of zinc are strongly electropositive to the noble metals.[3] The clinical significance of this finding was not investigated, however, in respect to the possible decomposition of the cement. The most extensive investigation in this field was carried out by Schriever,[4] and it was upon his work that the specific approach to this research was based. His paper carries a very complete analysis of the literature.

It is apparent from the sparcity of scientific papers that much remains to be learned concerning the electric conductivity of cements, even though these materials are commonly employed in dentistry for the purpose of insulating the pulp. It is hoped that by establishing some basic units of conductivity, a better understanding and evaluation in the choice and use of dental cements can be made. The systems to be explained are the outgrowth of many experimental setups and are felt to be accurate and workable.

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PROCEDURE

Schriever[4] carefully measured the voltages which exist between dissimilar metals in the mouth. From these data he calculated the current which actually flowed as a result of these voltages. These currents are in the magnitude of 0.5 microampere. This also corresponds to work done by Reed and Willman.[5] The work of Schriever is fundamentally sound and a great advancement to a better

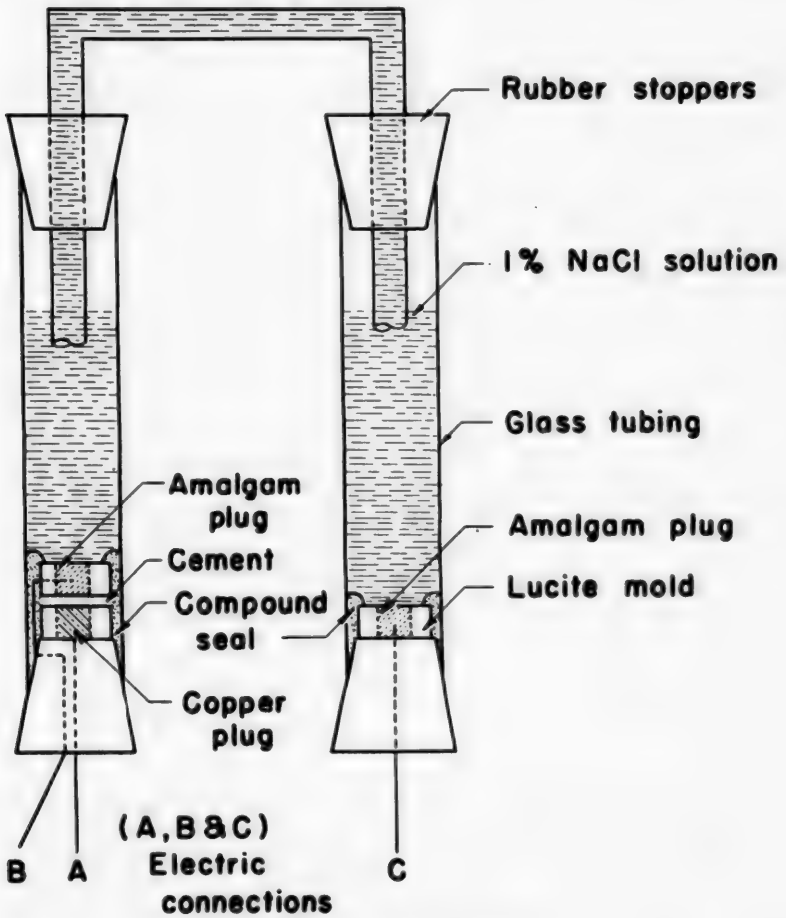

Fig. 1.—Basic cell first employed.

understanding of galvanic currents and their behavior in the mouth. However, no mention was made by Schriever as to whether cement bases were present under any of the restorations being tested; thus, the part that the cement base played in making up the aggregate resistance of the current circuit was not indicated. Knowing the approximate values of the currents which do flow in

the mouth, this experiment was designed to duplicate those currents in vitro and then utilize the test cells to investigate the insulating properties of the cementing material itself.

The cell that was first employed is illustrated in Fig. 1. A Lucite mold, 0.25 inch inside diameter, was packed with amalgam. A copper wire *(C)* was embedded in the amalgam to establish the electric contact. Another Lucite mold of the same dimensions was made with a copper plug insert. The copper was separated from an amalgam plug, similar to the one previously described, by a 1 mm. layer of zinc phosphate cement. Electric connections (*A* and *B*) were made to these plugs and brought out through the end of the cell. The cells were then sealed from the electrolyte with the exception of the exposed metal surfaces; thus the electrolyte did not come in contact with the cement itself. The 1 per cent sodium chloride solution simulated the saliva and served as an electric circuit for the cells. This cell is basically similar to one used by Schoonover and Souder[2] in their work on corrosion. The bone fluid resistance, that is, a term used by Schriever for dentin, soft tissue, alveolar bone, and so forth, was simulated by using an external resistance. The current was adjusted to approximately 0.5 microampere, as found by Schriever in his clinical measurements.

In this experimental work it was immediately found that actually two distinct cells exist, one between A and B and one between B and C. Thus the current that flowed was due to a resultant voltage of the two different cells. The cement itself is electropositive to the amalgam; therefore, there is created a cell between the cement and the cement and the amalgam, with the cement being the positive electrode. The copper plug served as an electric connection to the cement. In the majority of these cells the polarity of the C amalgam was positive with respect to the B amalgam. This meant a bucking voltage, and the resultant voltage was then the difference between the two. In all cases AB voltage exceeded the BC voltage, and consequently A was positive and C negative for the resultant voltage. This cell was valuable in acquiring some basic knowledge of the cement behavior and in establishing proper instrumentation. However, since the primary purpose of this study was concerned with the conductivity of the insulating medium itself rather than the relationship of the various cells created, it was found that more rapid progress could be made by utilizing only the AB portion of these cells.

It was imperative that the construction of the test cells be standardized. As with the first cell described, a Lucite mold of definite dimensions was used in the fabrication of the metal plugs. The Lucite served as an effective insulator. In Fig. 2 can be seen a mold packed with amalgam and a copper wire leading out for establishing the electric contact. A special die, Fig. 3, was employed in order to control the thickness of the cement. This die had a recession 1 mm. deep and when the Lucite mold was placed on the die, Fig. 4, the amalgam could be packed against this short post, leaving an area exactly 1 mm. in depth to be filled with cement. A tightly fitting copper plug was placed in another Lucite mold, Fig. 5. The electric connection to this part of

Fig. 2. Fig. 3.

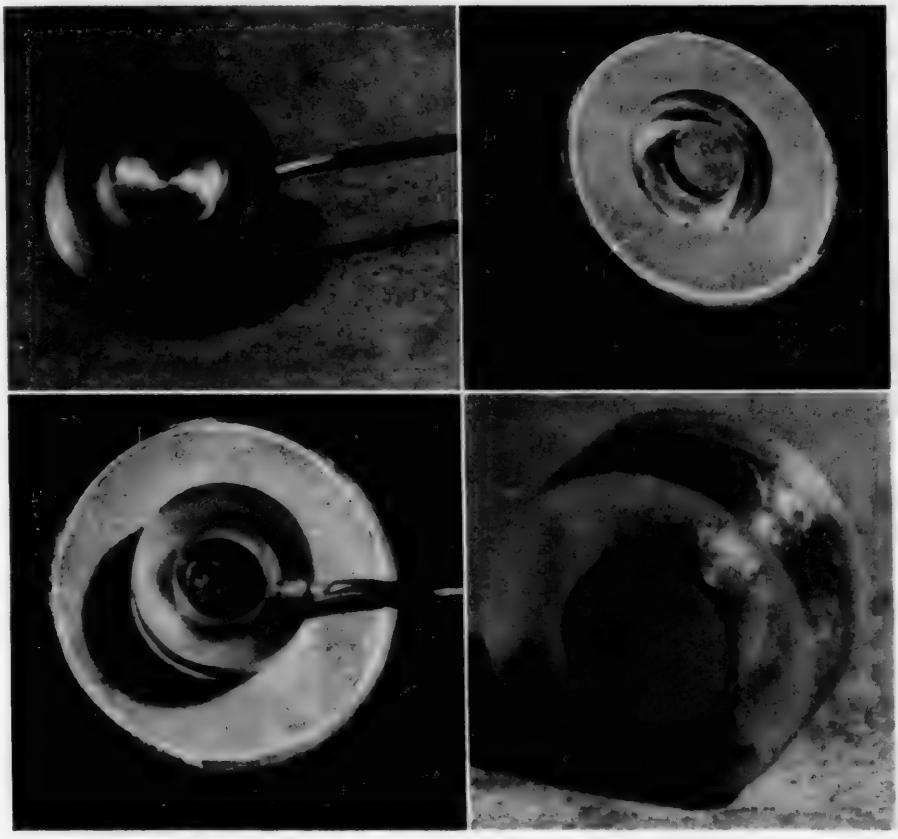

Fig. 4. Fig. 5.

Figs. 2-5.—Component parts of cell. Fig. 2, Amalgam plug with 1 mm. recession. Fig. 3, Die. Fig. 4, Lucite mold on die and electric connection in place. Fig. 5, Copper plug.

Fig. 6. Fig. 7.

Figs. 6 and 7.—Completed cell before and after sealing with compound.

the cell was made through a spring clamp. In order to avoid variations in contact pressure between the cement and the metal plugs, this device was used to maintain constant pressure. In Fig. 6 can be seen the clamp holding the finished cell. It was felt that for our purposes this exerted pressure would be sufficiently uniform for each cell. Fig. 6 shows a connection between the clamp jaws and the copper plug, thus providing the electric connection to the external circuit. It is evident that there was no contact between the clamp and the amalgam plug. The electric connection to this part of the cell was made through the copper wire. The entire cell was then sealed with compound, Fig. 7, to prevent dehydration of the cement.

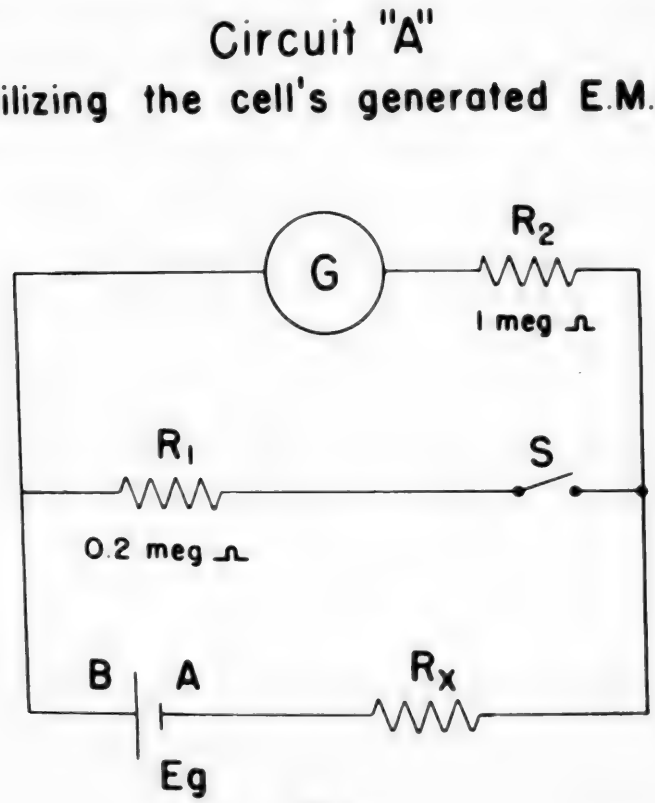

Fig. 8.

It was realized that metals other than amalgam and copper might be more comparable to the clinical conditions. However, this investigation was not concerned with the cells per se but only with the resistance of the cementing material itself. While the amalgam-cement relationship was a close approach to the clinical use of these materials, the copper-cement relationship served only as a medium of contact. The actual contact of cement with the

dentin and the other "bone fluid" circuit which exists in the mouth would, of course, be difficult to duplicate in the laboratory. Future work will be conducted using other metals, such as gold, in place of copper. It is felt, however, that while the different metals may affect the magnitude of the generated voltage of the cement-metal interface, the basic resistance of the cement itself would be affected very little.

In order to avoid drawing excessive current from this cell without altering the circuit constants, and yet accurately measure these constants, two circuits were designed. In Fig. 8 can be seen the diagram for Circuit A. The measuring instrument *G* has an input impedance, R_2, of 1 megohm. This instrument was utilized as a voltmeter in order to measure cell potential. *Eg* represents the voltage of the cell which is being measured and its unknown resistance, R_x. The additional circuit of R_1 and *S* serves to provide the readings for an additional equation which is needed to calculate R_x. The necessary derivation of the formulas employed for calculating the resistance of the cementing material is shown as follows:

V_1 = reading of G with S open
V_2 = reading of G with S closed
I_1 = current flow through cell with S open
I_2 = current flow through cell with S closed
Eg = generated voltage of cell
Rx = resistance of insulating medium
R_2 = input impedance of G

$$I_1 = \frac{Eg}{Rx + R_2} \text{ and } V_1 = I_1 R_2$$

By transposing:

$$I_1 = \frac{V_1}{R_2}$$

Equating the two equations for I_1: $\frac{Eg}{Rx + R_2} = \frac{V_1}{R_2}$ and transposing

(1) Therefore:

$$Eg = \frac{V_1 Rx + V_1 R_2}{R_2}$$

Now:

$$I_2 = \frac{V_2}{R_2} \times 6 \text{ since } R_2 = 5\ R_1$$

And:

$$I_2 = \frac{Eg}{Rx + \frac{R_1 R_2}{R_1 + R_2}}$$

Since:

$$\frac{R_1R_2}{R_1+R_2} = 0.167 \text{ megohm},$$

$$I_2 = \frac{Eg}{Rx + 0.167 \text{ megohm}}$$

Equating the two equations for I_2: $\dfrac{Eg}{Rx + 0.167 \text{ megohm}} = \dfrac{6V_2}{R_2}$

(2) Therefore:

$$Eg = \frac{6V_2Rx + V_2}{R_2}$$

Equating (1) and (2): $\dfrac{V_1Rx + V_1R_2}{R_2} = \dfrac{6V_2Rx + V_2}{R_2}$

(3) Therefore:

$$Rx \text{ (megohms} = \frac{V_1R_2 - V_2}{6V_2 - V_1}$$

The derivation is quite simple and the formula for Rx shows that the measured values V_1 and V_2 are the critcial readings.

This circuit did, of course, utilize the generated electromotive force (E.M.F.) from the cement-amalgam and cement-copper interfaces to produce the current flow in the circuit. Because of the low values of current which result as the cell ages, there are limitations to the magnitude of resistance which can be determined in this type of circuit. Thus it was felt that an additional circuit design, Circuit B shown in Fig. 9, might be utilized to measure these higher values and to substantiate the measurements obtained with Circuit A. The measuring instrument, *G*, has a limiting resistance, R_1 plus R_2, in series with it. In this circuit it is used as an ammeter, in reality a microammeter. The external source of E.M.F. is represented by *Es*. The cell being investigated is represented by *Eg* and its internal resistance is R_x. The double-pole double-throw switch allows reversal of the polarity of *Es* with respect to *Eg* and thus provides necessary data for setting up simultaneous equations in order to calculate R_x. In one position, the cells are series-aiding and in the other they are series-bucking. The circuit was so designed that the current through the cell never exceeded 1 microampere. The derivation of the formulas for computing the cement resistance in this circuit is shown as follows:

I_1 = current flow when E.M.F.'s are bucking
I_2 = current flow when E.M.F.'s are aiding
Es = external source of E.M.F.
Eg = cell E.M.F.
$R_2 + R_1$ = limiting resistance, 1 megohm each
Rx = resistance of insulating medium
S = double-pole, double-throw reversing switch

Ohm's law shows that

$$\frac{E}{I} = R$$

Therefore: $$Rx + R_2 + R_1 = \frac{Es - Eg}{I_1}$$

(1) Transposing: $$Rx = \frac{Es - Eg}{I_1} - 2 \text{ megohms since}$$

$$R_2 + R_1 = 2 \text{ megohms}$$

Now in order to obtain the second of a pair of simultaneous equations, the cells are reversed so that they are aiding instead of bucking. This is done simply by reversing S.

So: $$Rx + R_2 + R_1 = \frac{Es + Eg}{I_2}$$

(2) Transposing: $$Rx = \frac{Es + Eg}{I_2} - 2 \text{ megohms since}$$

$$R_2 + R_1 = 2 \text{ megohms}$$

Equating (1) and (2): $$\frac{Es - Eg}{I_1} - 2 \text{ megohms} = \frac{Es + Eg}{I_2} - 2 \text{ megohms}$$

Or: $$EsI_2 - EgI_2 = EsI_1 + EgI_1$$

Transposing: $$EgI_1 + EgI_2 = EsI_2 - EsI_1$$

And: $$Eg = \frac{Es\ (I_2 - I_1)}{I_2 + I_1}$$

(3) Substituting in (1): $$\boxed{Rx \text{ (megohms)} = \frac{Es - \frac{Es\ (I_2 - I_1)}{I_2 + I_1}}{I_1} - 2 \text{ megohms}}$$

Here again the critical values are the current readings I_2 and I_1. As will be shown in the following data, this circuit did give comparable results to Circuit A. By using a flashlight battery and a fixed resistance (manufacturer marking, 75,000 ohms) in series, the amalgam-cement cell was simulated. Using the A circuit the values were as follows:

$V_1 = 280$
$V_2 = 207.5$
$Rx = 75{,}100$ ohms using formula (3) of A circuit

Then connecting this cell into the B circuit, the readings were as follows:

$I_1 = 45.5$
$I_2 = 337.5$
$Rx = 75{,}000$ ohms using formula (3) of B circuit

Now taking a typical cell, one of the original types that we used, and placing it in A circuit, the readings were:

$V_1 = 63$
$V_2 = 46$
Rx = 79,800 ohms formula (3)

With the B circuit the readings were:

$I_1 = 163$
$I_2 = 219$
Rx = 79,600 ohms formula (3)

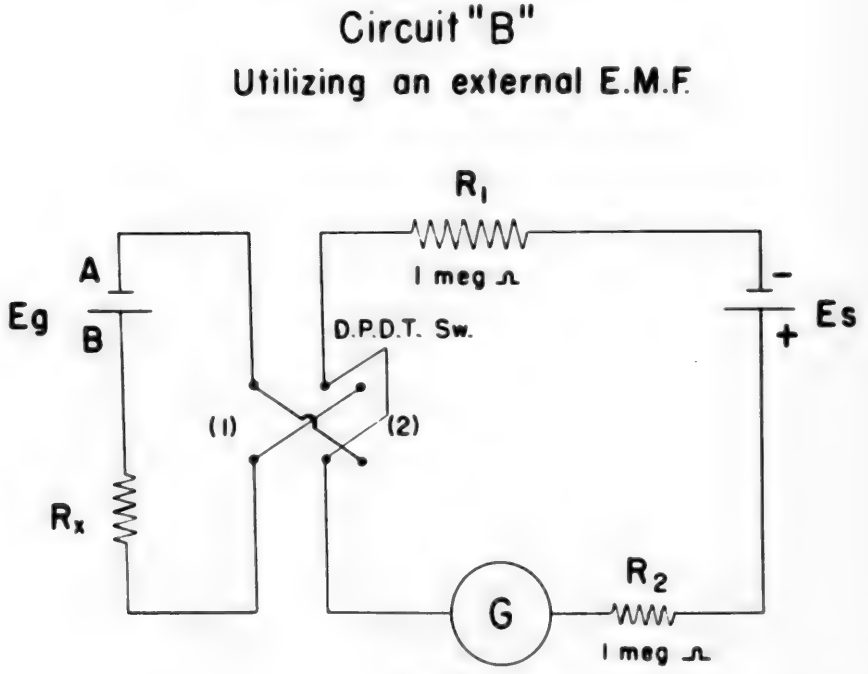

Fig. 9.

From this information it is obvious that the two circuits produced equally accurate readings. In the first case the known resistance checked in both circuits, with a difference of less than 0.14 per cent between each other as well as from the stated value of resistance. In the second case the two circuits differed by slightly more than 0.25 per cent. This is well within the limits of the experimental error.

The galvanometer employed in both circuits was a Leeds-Northrup high sensitivity galvanometer with a sensitivity of 5.15 microvolts per millimeter. This instrument was adapted so that its input impedance was 1 megohm, and consequently the circuit constants were affected very little. A further check

on the instrumentation, by use of a high input impedance (11 megohms) vacuum tube voltmeter, indicated comparable accuracy with the former instrument. Thus this galvanometer, with a current sensitivity of 0.00515 microampere per millimeter, was equally adaptable in either of the two circuits utilized.

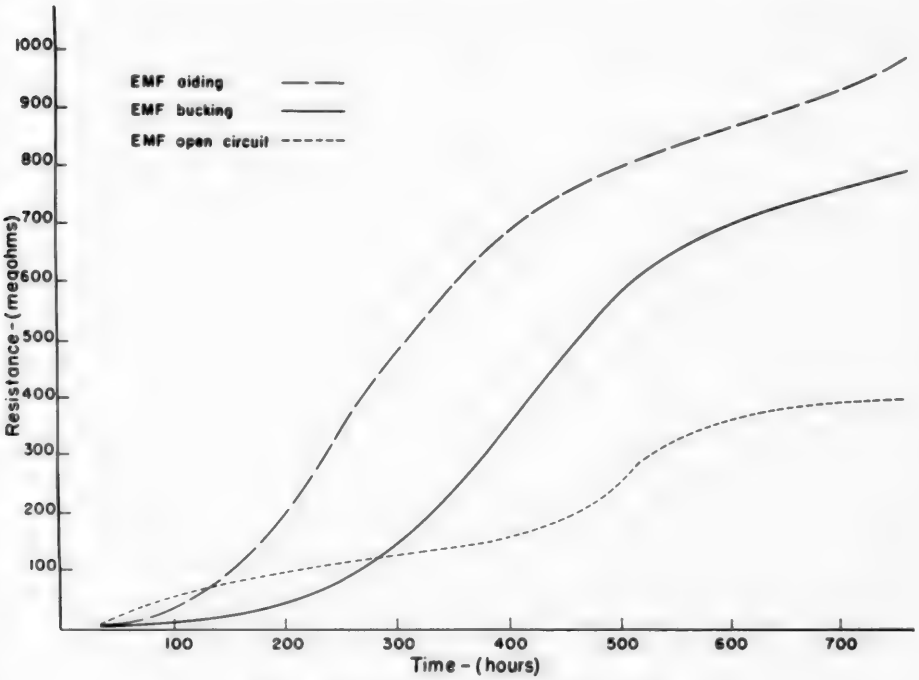

Fig. 10.—Variation of resistance with time.

In taking readings, the procedure was standardizd by closing the circuit for one-half minute, taking a reading, opening the circuit for one minute, then closing the circuit for reversed polarity (as explained in the B circuit formula derivation), and waiting one-half minute before reading again. It was soon found that polarization at the cement-metal interface was an important factor and dependent on both the time and magnitude of current flow. Thus it was important that these time intervals be standardized because of the effects of this polarization.

Table I shows some of the data that have been collected. The same batch of cement and same powder-liquid were used in all cells. The time column indicates the time, after assembly of the cell, that the reading was taken. These cells were all placed on a closed circuit following the first reading. The current was controlled and initially set at approximately 0.5 microampere. The B circuit was used and the source E.M.F. was connected series-bucking. There were ten cells and the time of the intial reading of each was staggered.

This was in an attempt to determine whether the actual reading of the cell played any important part in its resistance pattern. It was found that it did not. It will be noted that the resistance increased with time in Cells I through X prior to the first reading of each cell. Cell I read 0.17 megohm while cell X read 20.85 megohms. The increased resistance can probably be attributed to completion of the chemical reaction within the cement. These data seem to indicate that the polarizing effect produced when the circuit is closed is not the primary cause of increasing cell resistance.

TABLE I

CIRCUIT B: CELLS ON CLOSED CIRCUIT AFTER FIRST READING E.M.F.'S AIDING TIME—RESISTANCE CHART. AMALGAM—ZINC PHOSPHATE—COPPER CELLS.

TIME OF READING AFTER ASSEMBLY (HR.)	CELL NUMBER AND RESISTANCE IN MEGOHMS									
	I	II	III	IV	V	VI	VII	VIII	IX	X
¼	0.17									
½	0.22	0.24								
1	0.17	0.22	0.24							
2	0.30	0.23	0.24	0.28						
4	0.34	0.44	0.34	0.36	0.42					
8	0.54	0.58	0.52	0.56	0.61	0.63				
16	0.90	1.46	1.21	1.17	1.66	0.93	1.20			
32	1.51	3.92	2.68	3.80	4.84	1.86	4.06	1.51		
64	2.80	8.25	5.00	10.12	5.33	4.89	14.00	15.80	7.55	
128	2.35	6.72	5.27	9.11	3.27	3.34	38.00	18.00	21.50	20.85
256	55.00	16.00	26.50	48.00	46.60	3.22	87.00	264.5	92.00	198.0
384	31.40	47.40	112.0	208.0	20.70	16.70	362.0	1000+	570.6	1000+
512	64.60	86.90	226.0	400.0	40.00	Opened	Opened	Opened	1000	Opened
765	103.0	134.0	340.0	1000	56.30				Opened	
	Opened	Opened	Opened	Opened	Opened					

If one examines the behavior of a single cell, for example Cell I, while on closed circuit, it can be seen that the resistance also increased with time. Up to sixty-four hours the resistances of the cells were approximately the same. After sixty-four hours the values were more variable, although in general they increased. The variation in resistance between individual cells can probably be attributed to cracking of the cement, as was found when some of the cells were opened. The picture, however, is one of increasing resistance with time. When the resistance reached 1,000 megohms, the tests were discontinued, since higher values would be beyond the range of our equipment, and furthermore a value of resistance has already been reached to indicate that zinc phosphate cement is an effective insulator to electric energy under the experimental conditions that have been investigated.

Table II is a similar chart with the only difference being that the source E.M.F. is connected in the B circuit series-aiding instead of -bucking. This of course would reverse the direction of current flow through the cell since the source E.M.F. is the greater voltage. The direction of current flow had little effect on the resistance picture. The rate and direction of change of resistance are very similar to those shown in Table I. There is one point to be

noted. When the E.M.F.'s are series-aiding, the magnitude of current through the cell is greater. The polarizing effect of the current would of course be greater in the circuit where the E.M.F. is aiding. These data are summarized in the graph in Fig. 10, where the magnitude of the resistance for a given time is greater in the series-aiding circuit as compared to the series-bucking one. Nevertheless, they both reach high values of resistance after sufficient time has elapsed. The actual differences here may just be academic and not of any significance. The values reached indicate that the cement is an effective insulator in both groups of cells under the conditions present.

TABLE II

TIME—RESISTANCE CHART. AMALGAM—ZINC PHOSPHATE—COPPER CELLS.
CIRCUIT B: CELLS ON CLOSED CIRCUIT AFTER FIRST READING (E.M.F.'S AIDING)

TIME OF READING AFTER ASSEMBLY (HR.)	CELL NUMBER AND RESISTANCE IN MEGOHMS									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1	0.24									
2	0.28	0.21								
4	0.59	0.78	0.48							
8	1.39	1.36	0.82	0.74						
16	2.27	3.55	1.70	1.15	1.06					
32	8.68	10.11	3.34	2.82	4.90	3.80				
64	11.35	11.40	4.05	4.05	5.55	7.86	26.15			
128	48.00	38.00	16.60	7.64	20.20	28.80	139.0	39 00		
260	600.0	600.0	123.0	50.00	200.0	325.0	800.0	264.0	264.0	
384	650.0	1,000	175.0	75.00	316.0	661.0	1,600	886.0	665.0	614.0
512	800.0	1,000+	200.0	100.0	400.0	1,000	Opened	1,140	800.0	665.0
750	1,000	Opened	664.0	570.0	1,000+					

TABLE III

TIME—RESISTANCE CHART. AMALGAM—ZINC PHOSPHATE—COPPER CELLS.
CIRCUIT B: CELLS ON OPEN CIRCUIT EXCEPT WHEN MEASURED

TIME OF READING AFTER ASSEMBLY (HR.)	CELL NUMBER AND RESISTANCE IN MEGOHMS								
	I	II	III	IV	V	VI	VII	VIII	IX
1		0.22							
2		0.42	0.48						
4		0.58	0.66	0.74					
8		0.84	0.69	0.83	0.51				
16		1.62	1.18	1.61	0.84	1.41			
32		4.70	1.83	6.44	1.78	3.50	1.28		
64		17.55	3.22	20.55	5.12	19.20	19.16	20.05	
128		50.50	12.60	45.80	16.35	63.80	43.10	58.60	45.80
256		129.0	35.40	96.30	48.70	155.0	103.0	129.0	96.30	64.60
384		260.0	58.50	133.5	76.50	260.0	162.0	138.0	149.0	110.0
512		615.0	114.0	260.0	126.0	397.0	308.0	238.0	246.0	204.0
768		1,024	274.0	324.0	166.0	524.0	493.0	250.0	282.0	264.0
1,200		1,703	798.0	525.0	259.0	886.0	1,118	377.0	447.0	460.0

In Table III can be seen data obtained from similar cells placed on open circuit except during the time interval while the cell was being read. This is a further check on the effect of the current on the resistance pattern. The

same resistance pattern is present that was found in the other two charts. There is an increasing resistance with time and, as seen from the data and also Fig. 10, the values of resistance reached with these were approximately the same as those found in the closed circuit method. The polarizing effect of the current on these sealed cells seems to have little effect on the rate or manner in which the resistance changes. It is important here to emphasize that these cells are sealed and dry, and whether or not this condition exists in cells where the cement is maintained in a moist condition remains to be investigated.

DISCUSSION

It is difficult to interpret these data in terms of the cement resistance under actual clinical conditions. All of the tests to date have been conducted with the cement sealed against loss or gain in moisture. Naturally, a cement base, or the thin layer of cement surrounding a gold inlay, is not as dry as these specimens. The actual amount of moisture or fluid to which the cement is exposed in the cavity preparation is, of course, unknown. However, a series of new cells is being tested in such a manner that the cement margin can be exposed to normal saline solution and also distilled water. The resistance pattern may be quite different in these cells. It is to be noted that this is still not the actual clinical picture, but merely two extremes. The absence of moisture in the first group of cells produced high values of resistance. It is felt that the group of cells with exposed margins may produce much lower values of resistance. The clinical picture should be one where the resistance falls between these two limits. The degree of moisture present and whether it is a base for an amalgam restoration or the seal for a gold crown or inlay would be variables that determine just where between these two limits the resistance will fall. Nevertheless, this phase of the study was designed primarily to establish a workable system for measuring electric conductivity, and the preliminary tests were conducted on dry specimens in order to control better the variables associated with the operation of the cells.

SUMMARY

There is available no basic information on the electric conductivity of dental cements and the influence played by such variables as composition, liquid-powder ratio, and thickness. This has been a discussion of the instrumentation, circuit design, and cell construction which have been developed for accurately measuring the electric conductivity. A few preliminary experiments were summarized to show the resistance pattern on aging of the cement specimen. No attempt should be made at this stage to interpret the practical significance of these data in terms of the cement's resistance to electric energy. Further tests under different conditions will be required.

We wish to acknowledge the valuable assistance of Mr. Robert Johnson, who assisted in the preparation of many of the specimens.

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DENTAL RESTORATIVE MATERIALS: AMALGAMS, ACRYLICS

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AMALGAM is by long odds our most important filling material. Brekhus,[1] observing fillings in 422,952 teeth in 1929, found nearly 70 per cent of them to be amalgam, and only 10 per cent of them needed replacing. This was the lowest percentage failure of any of the types of fillings observed. A survey made for a manufacturer two years ago revealed that about 80 per cent of all fillings were amalgam.[2] Little change has occurred in at least three popular alloys in the last twenty years (Table I). These are the results of tests made on alloys in the years indicated. The most significant change noted over this period in about twenty certified alloys is that the directions for use which came with the alloys in 1952 cautioned against moisture contamination. Probably, it is easier to add this caution in the directions than to improve metallurgic procedures to produce good zinc-free alloys.

The porous unetched surface of an amalgam filling magnified 100 times is shown in Fig. 1. The porosity is probably due to pools of free mercury trapped during the packing and leaving voids after the chemical reaction was complete. The structure and density of this filling appear to leave much to be desired, most of which could be accomplished by the removal of more mercury. The appearance of this filling is not much worse, if any, than most amalgam fillings. It was rendering good service and had been for a long time, as were others analyzed for mercury content (Table II).

Mercury content varied from 59 to 45.2 per cent, with only one filling below 49 per cent. Any dentist, using an alloy which would pass American Dental Association specifications for amalgam, would have great difficulty in condensing a filling so well that he would have less than 47 per cent mercury. Therefore, No. 2 probably was an alloy high in tin. Souder[3] reported on two fillings of long service, having 51.6 and 52.9 per cent mercury. Phillips[4] reported on 100 fillings selected at random, and found that the Hg content varied from 28.6 to 61.0 per cent, with an average of 45.4 per cent.

Ward and Scott,[5] working with alloys which met the American Dental Association specifications, obtained 47.47 per cent Hg with their heaviest packing pressure and 59.89 per cent Hg when amalgam was jarred into mold.

Our results using an alloy which meets American Dental Association specifications are shown in Table III. They agree with those of Ward and Scott very closely. On the basis of these data, it would appear that there was some other reason than manipulation responsible for the low Hg content in Phillips' 100 fillings.

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Since the mercury content of the amalgam was about 60 per cent when it was only jarred into the mold, and since both Phillips' data and ours show approximately this much mercury in the fillings, it is difficult to refrain from an obvious observation that explains the thumb- or fingerprints on surfaces of amalgam fillings. Only very light pressure on a 2 mm. plugger brings the Hg content down to 53 per cent and light pressure to 50 per cent. Perhaps it would be asking too much to hope for at least light pressure in packing amalgam fillings. However, this may not be necessary, for at least the ten fillings that we tested and the two that Souder analyzed averaged higher than 50 per cent Hg content. Souder knew that those fillings had been in service for over ten years. The evidence from satisfactory amalgam fillings could indicate that strengths which correspond to those obtained with 52 per cent Hg are ample, assuming there is some correlation between strength and mercury content. Ward and Scott[5] found that mercury content decreased and crushing strength increased as packing pressures increased. At 256,000 psi packing pressure he obtained 28.3 per cent Hg and 73,000 psi crushing strength.

TABLE I

SETTING REACTION, FLOW, AND STRENGTH OF THREE POPULAR AMALGAMS AT TEN-YEAR INTERVALS

ALLOY	EXPANSION (MICRONS PER CM.)			FLOW (%)			ULTIMATE STRENGTH (PSI)		
	1932	1942	1952	1932	1942	1952	1932	1942	1952
1	4	3.7	6.0	2.2	2.0		52,600	52,500	53,500
2	6	6.5	4.3	2.1	3.0		53,700	49,000	59,500
3	9	2.1	7.4	5.3	3.2		40,000	47,000	51,000

TABLE II

MERCURY CONTENT OF TEN AMALGAM FILLINGS, ALL HAVING RENDERED SATISFACTORY SERVICE

MERCURY CONTENT OF FILLINGS FROM EXTRACTED TEETH IN PER CENT	
1. 50.4	6. 50.4
2. 45.2	7. 58.6
3. 49.2	8. 49.6
4. 57.4	9. 59.0
5. 52.5	10. 52.4
Average 52.47	

TABLE III

EFFECT OF PACKING PRESSURE ON REMOVAL OF MERCURY FROM AMALGAM SPECIMENS

POUNDS OF PRESSURE PER SQ. IN.	EQUIVALENT NUMBER OF POUNDS ON 2 MM. PLUGGER		PERCENTAGE OF MERCURY
None	None	Jarred into mould	60
500	2.40	Very light hand pressure	53
1,000	4.88	Light hand pressure	50
2,000	9.74	Heavy hand pressure	48
3,000	14.64	Very heavy hand pressure	47–
Pressures below are beyond present clinical possibility			
4,000	19.48	----------	45+
5,000	24.40	----------	44
10,000	48.80	----------	40
20,000	97.40	----------	35

Gray's[6] correlation between Hg content and strength is transposed in part and shown in the dotted lines in Fig. 2. As packing pressure increases, mercury content goes down and strength goes up at a very rewarding rate. Our results, shown in solid line (Fig. 2), are similar, but the rewards for reducing mercury content beyond 5,000 psi packing pressure are far less. We do not seem to be able to produce striking increases in strength after packing pressure which brings the mercury content to about 44 per cent. Plugger pressures which correspond closely to 3,000 psi are possible and will produce strengths of very respectable amounts. Possibly, the effort required to reduce mercury content below 47 per cent is hardly worth the slight increase in strength gained. However, since there are fastidious and conscientious operators who are hungry for clues that will make possible some improvement, even though slight, it becomes our duty to report anything that might be helpful.

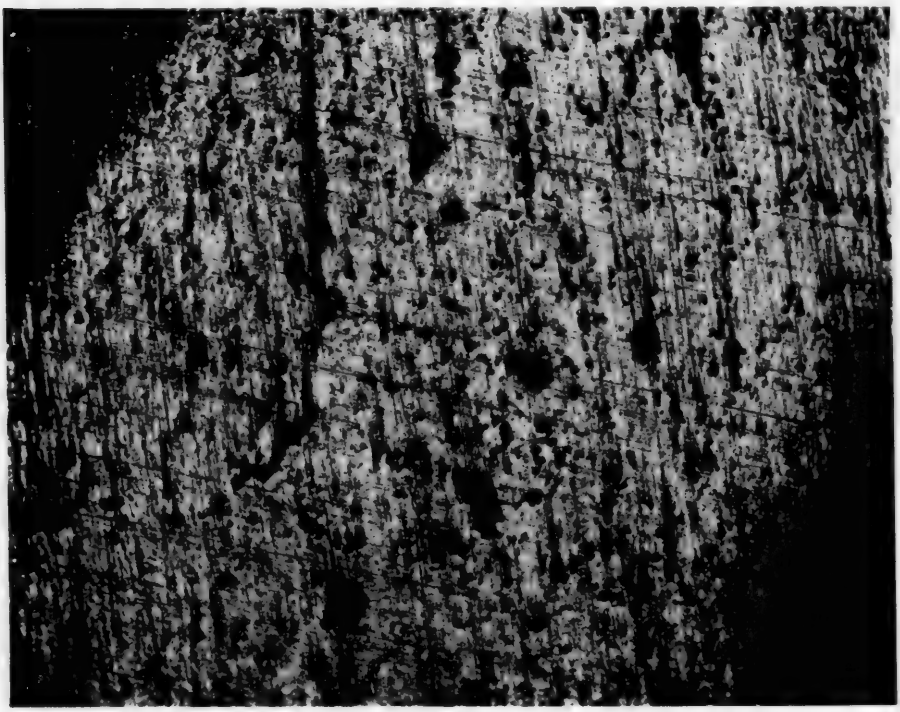

Fig. 1.—Photomicrograph of unetched section of successful amalgam filling. (Magnification ×100.)

Since we were intrigued by the thought that a pancake of amalgam (Fig. 3) could be quickly made by removing the mercury under high pressure (about 5,000 psi) and then vibrating pieces of the alloy into the mold or cavity with a mechanical condenser (Fig. 4), the following experiment was set up:

Three commonly used alloys were selected, and the following nine methods of mixing and packing were to be used (Figs. 5, 6, and 7): (1) The alloy was

to be weighed, mixed, and packed as directed by manufacturer. This was to be the control for each other method of mixing and packing. (2) The mix was the same but the packing was to be done with the mechanical vibrator. (3) The mix was the same, and a pancake was made under 5,000 psi and pieces from it were vibrated in the mold. (It should be noted here that this procedure did not work out as hoped. The dry pieces of amalgam set too fast and refused to unite under the vibrator. Only by starting out with a small piece of amalgam from which the mercury was not expressed was it possible to get the dry pieces to unite.) (4, 5, and 6.) The amalgams were packed the same but mixed longer in hope of further reducing mercury content and increasing both early and ultimate strength. (7, 8, and 9.) The amalgams were packed the same but mixed with the Wig-L-Bug for approximately ten seconds.

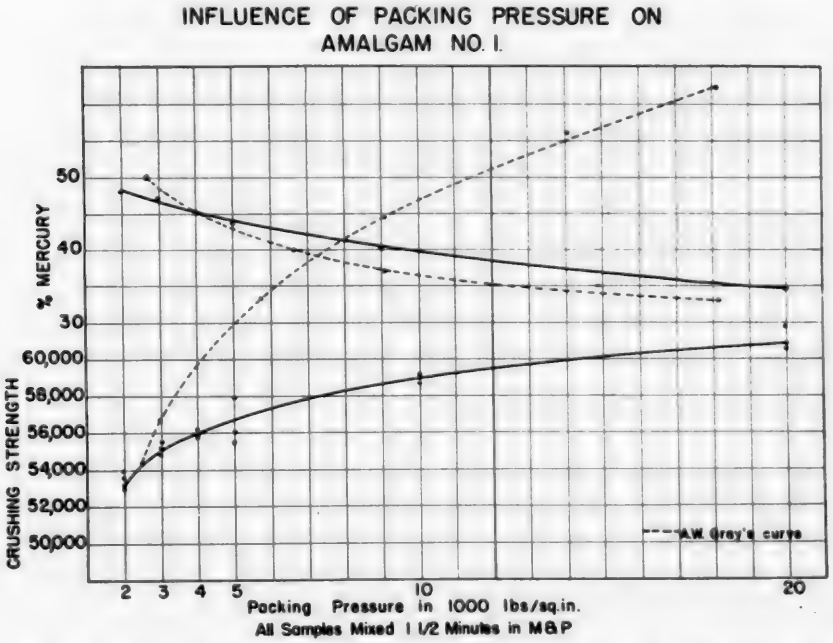

Fig. 2.—Effect of mercury removal on strength of amalgam. All specimens made by both Gray and us were prepared by using mechanical packing pressure indicated on a plugger same size as mold.

The results followed a pattern for each alloy but, of course, the properties of the individual alloys varied to a certain degree. Alloy No. 1 (Table IV) shows little important change with all these different technics of mixing and packing. The pancake idea seemed to show some slight promise in the shorter mixing technic (Mix 3 as compared to 1 and 2). However, this was not true for the longer mortar mix or mechanical mix. Comparing hand packing with mechanical packing, there were no significant differences in strength or mercury content (Mixes 1 and 2, 4 and 5, or 7 and 8). This is not to say, however, that

mechanical condensation would not benefit fillings placed in the mouth. Condensing amalgam with finger pressure in the mouth as compared to a mold on a bench are two quite different problems.

Fig. 3.—Amalgam ready for packing, having had mercury expressed under 5,000 pounds per square inch pressure.

Fig. 4.—Mechanical condenser used in these tests.

Longer trituration and mechanical mixing resulted, as would be expected, in an increase in strength and a decreased expansion. It was disappointing that the mercury content did not correlate better with strength and dimensional changes. There seemed to be no correlation with this amalgam or with the other

two amalgams tested. There is a definite relationship with the data in Fig. 2, but in those studies only packing pressure was changed, whereas in the study illustrated by Table IV other factors known to have an influence on strength were changed. It must be deduced that strength cannot be predicted by a knowledge of Hg content alone.

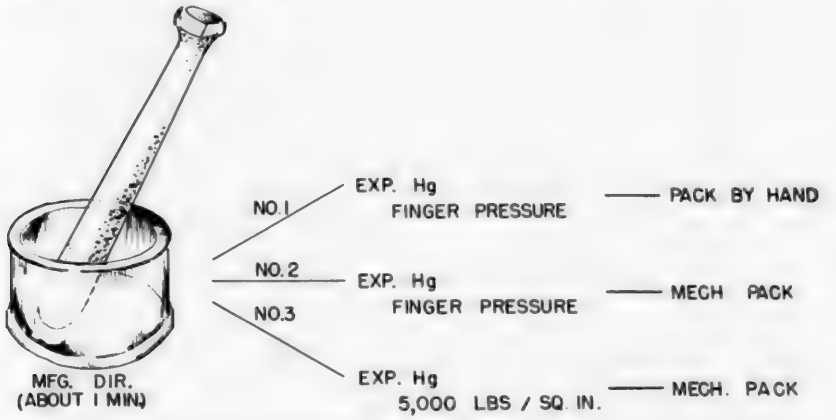

Fig. 5.—Method used in mixing and packing amalgam in three of the test methods.

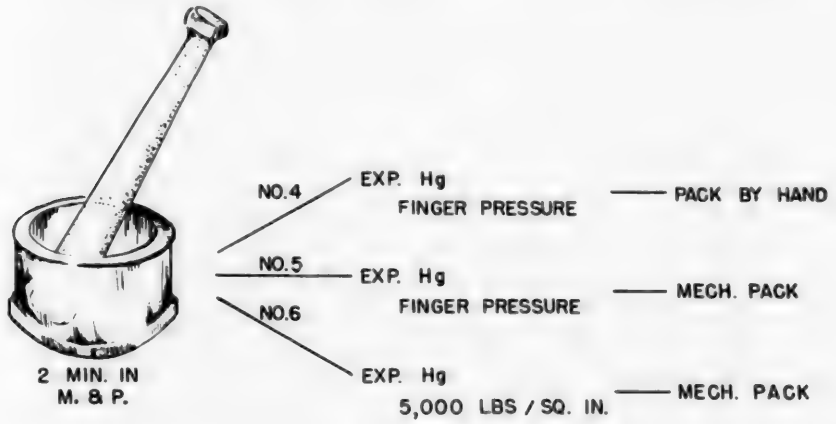

Fig. 6.—Method used in mixing and packing amalgam in three of the test methods.

Mixes 1 and 2 were triturated one minute each, whereas 4 and 5 were triturated two minutes each (1 and 4 were packed by hand, 2 and 5 were packed with a mechanical condenser). Strength in one hour has increased 30 per cent with the longer mix, with a significant ultimate increase. The early strength is particularly important. However, the setting changes of the longer mixes show a little contraction, and the argument that an expansion of 3 to 13 microns per centimeter as a specification requirement is essential for a good filling would rule out this longer mix.

Phillips, Boyd, Healey, and Crawford[7] have shown that no differences can be observed in the quality of amalgam fillings, some of which contracted and some expanded well beyond the present specification limits. There was no indication of flow in these fillings even though the technic used in mixing and packing produced flow well above the upper limits of the maximum figure for flow (4 per cent) in the specification. Neither was there any recurrent caries around the fillings which contracted a little. With evidence to indicate that contraction of the order shown for Mix 4 and 5 is not harmful, and nothing but speculation to indicate that it is harmful, the wise clinician would do well to choose technic No. 4 or 5 rather than Technic 1 or 2. In subjecting plugs of this alloy to the air pressure test, higher pressures before leakage were obtained with Technic 4 than with Technic 1. In No. 1 some air oozed through the body of the plug. This can be easily understood if the structure were the same as in Fig. 1.

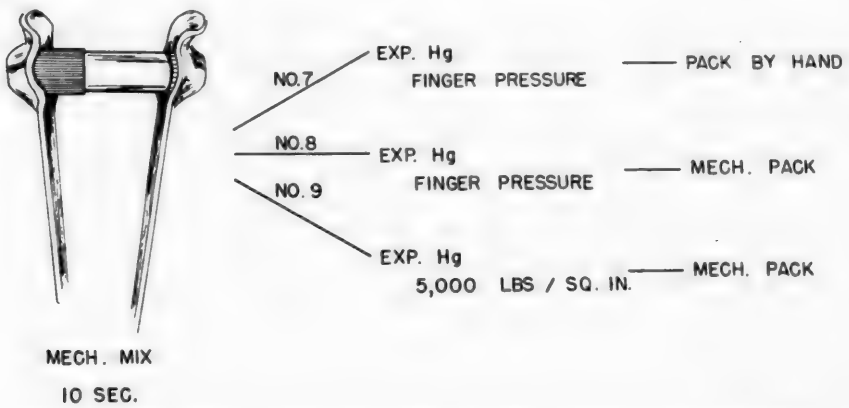

Fig. 7.—Method used in mixing and packing amalgam in three of the test methods.

It is unlikely that expansion of tooth structure results from amalgam expansion for any length of time, if at all. If this were true, the huge expansions resulting from moisture contamination would split teeth, and this has not been noted by clinical observation. Rather, the amalgam pushes itself out of the cavity. Setting expansion in amalgam occurs for the most part during the first three hours, which is the time allowed to permit amalgam to gain enough strength to support the static flow load. It is, therefore, difficult to conceive that expansion, occurring during this weak period, will expand tooth structure permanently. Much more needs to be understood about the effect of setting expansions as regards sealing cavities. Ignoring flow, there may be some logic that expansion in a one-surface filling would cause pressure that might result in further tightness at cavity walls, but the same expansion would be just as certain to cause looseness, if anything, in a three-surface filling. It is difficult to conceive expansion across the occlusal of an MOD filling, making it tighter at the mesial and distal proximal axial walls. In the early days

of inlay making and inadequate compensation for gold contraction, the contracted inlay fit so tightly at proximal axial walls that it was difficult, if not impossible, to seat it. Expansion made the inlay larger and looser, not tighter.

Fig. 8.

Fig. 9.

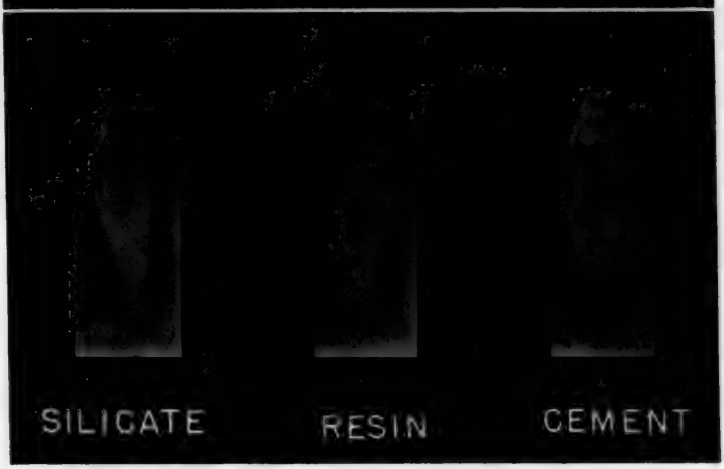

Fig. 8.—Radioautographs showing penetration around three types of filling materials.
Fig. 9.—Radioautographs showing penetration around fillings.

Table V shows results of the nine different mixing and packing technics on alloy No. 2. There is even less variation in the properties for this alloy than for alloy No. 1, and again no correlation with mercury content. This alloy is surprisingly resistant to change in properties with change in manipulation. Table VI shows the data for alloy No. 3. Setting reaction and strength show little change with varying mixing and packing technics.

TABLE IV

PROPERTIES OBTAINED FOR ALLOY NO. 1 USING NINE DIFFERENT TECHNICS

MIX	SETTING REACTION	STRENGTH (LBS./SQ. IN.) 1 HOUR	STRENGTH (LBS./SQ. IN.) ULTIMATE CRUSHING STRENGTH	Hg CONTENT % 1
1	+6	10,000	53,500	47.1
2	+5.5	9,000	50,000	47.6
3	+2.7	10,500	58,000	46.5
4	−2	13,000	61,000	47.4
5	−1.9	11,500	61,000	48.1
6	−1.4	12,500	58,000	47.3
7	0	8,500	56,000	47.2
8	+0.5	6,500	57,500	48.8
9	+0.8	9,000	55,500	47.1

TABLE V

PROPERTIES OBTAINED FOR ALLOY NO. 2 USING NINE DIFFERENT TECHNICS

MIX	SETTING REACTION	STRENGTH (LBS./SQ. IN.) 1 HOUR	STRENGTH (LBS./SQ. IN.) ULTIMATE CRUSHING STRENGTH	Hg CONTENT % 1
1	+4.3	17,000	59,500	46.5
2	+4.3	13,000	55,500	49.6
3	+4.9	13,000	53,500	48.4
4	+0.5	17,000	58,000	48.2
5	+4.4	14,000	56,500	49
6	+4.9	14,500	54,500	48.6
7	+3	15,000	58,000	47.4
8	+10	13,000	54,500	49.7
9	+4.6	15,500	55,000	49.4

TABLE VI

PROPERTIES OBTAINED FOR ALLOY NO. 3 USING NINE DIFFERENT TECHNICS

MIX	SETTING REACTION	STRENGTH (LBS./SQ. IN.) 1 HOUR	STRENGTH (LBS./SQ. IN.) ULTIMATE CRUSHING STRENGTH	Hg CONTENT % 1
1	+7.4	11,500	51,000	47.6
2	+7.6	10,000	48,000	49.4
3	+6.3	9,500	48,000	48.8
4	+0.5		52,500	49.2
5	+0.5		53,600	49.0
6	+0.82		51,000	49.1
7	+3		51,500	48.9
8	+5.5		51,000	50.6
9	+6.5		52,000	49.0

Armstrong and Simon[8] recently introduced a new tool for determining the tightness of fillings. Teeth with various types of fillings were inserted in solutions containing radioactive calcium. After varying periods of time, the teeth were mounted in plastic, sectioned through the fillings, polished, and

placed on sensitized film. It was found that where the solution had penetrated, the radioactive calcium would expose the film, and after development the film was dark or black where exposed. The surprising part of the early tests was that the solution penetrated around all kinds of fillings, more in the case of certain types of filling materials, nevertheless always. Follow-up studies by Robert Sausen[12] verified earlier findings. His results are shown in Figs. 8 and 9. The lines around the gold inlay, amalgam, and gold foil clearly show penetration of solution. The teeth were stored at room temperature. In Sausen's studies, as well as in Armstrong and Simon's,[8] the amalgam consistently produced the finest line, with gold foil showing not only surprisingly wide lines of penetration but penetration of the body of the filling. Penetration around the silicate filling (Fig. 9) can only be described as gross. This ease of penetration around silicate no doubt explains at least some of the reason that there is so much dissatisfaction with this clinically unsatisfactory filling material. Dentinal tubules seem to be wide open under silicates.

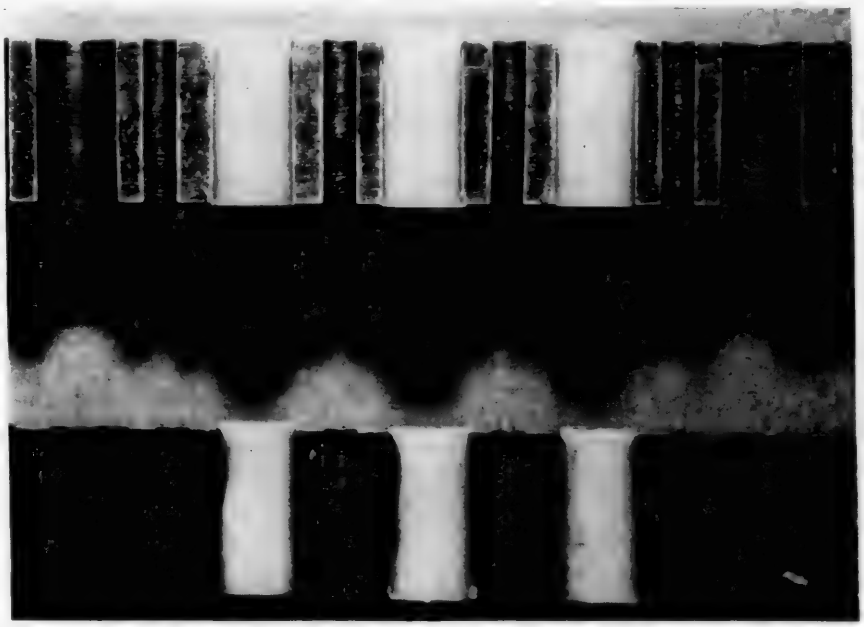

Fig. 10.—Split mold with plastic fillings showing contraction along sides of filling.

Figure 9 also shows penetration around a plastic filling. Resin fillings made by "brush-on" technic have always seemed reasonably tight with this test. It must be remembered, however, that these fillings were never in the oral cavity and subjected to rather wide temperature changes as described by Nelson, Wolcott, and Paffenbarger.[9] The plastic filling, to date, is not a clinical success. In spite of the manufacturer's claims, the plastic cement is not good for cementing inlays. According to Zander[10] and Seelig,[11] methyl methacrylate does not

injure pulp tissue. The disadvantages of this material are physical. It is too soft, it contracts so much on hardening that large voids develop about its walls, the contraction is so great that unless large undercuts are made the filling frequently will just fall out of the cavity, and no amount of matrix pressure can prevent these voids from occurring. Fig. 10 shows one-half of a split mold with plastic fillings in place. The samples at the bottom were formed using an excess of material, and as the material began to harden a glass slab was placed over it and heavily weighted. The flash seems quite tight, as is the case under a matrix on a tooth, but beneath the margin the shrinkage is apparent. The samples at the top were built up by small increments brushed on. This is a difficult and meticulous procedure. In small cavities it is difficult not to fill the whole cavity with the first bead. Even under ideal conditions in a short period of time, the results of fillings using this material are not very satisfying hygienically, physically, and aesthetically.

The acrylics at first seem to offer opportunities for developing into a better filling material. However, unless some very radical changes can be made in their properties, acrylics will not offer us an improved or satisfactory answer to the problem of replacing lost tooth structure.

A material not known to us now is needed to fulfill the requirements of a satisfactory filling material. We doubt that improvement in any of the materials or methods now employed, important though they may be, will provide a completely satisfying answer. The material we want will no doubt be found some day. The next step then is the same as the last—a search for a better filling material.

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A NEW INSTRUMENT FOR RECORDING ORAL MUSCLE FORCES: THE PHOTOELECTRIC MYODYNAGRAPH

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IMBALANCE of dental environmental muscle forces as an etiological factor in malocclusion has been widely discussed in orthodontics.[1-5] While it is a reasonable assumption that an imbalance between muscle forces of tongue, lips, and cheeks bears some clinical implication, scientific approach has been limited due to the lack of a suitable instrument for recording these muscle forces with precision and accuracy.

Friel,[6, 7] Lancet,[8] and, in recent years, Feldstein,[9] and Alderesio[10] have described various instruments for measurement of oral muscle forces. With the exception of the last,[10] on which there are not enough published data available, the other instruments do not meet the criteria set forth in this paper.

Requirements for an Instrument to Measure Oral Muscle Forces.—

1. The instrument should measure and record accurately the magnitude of muscle forces at rest, during function, and at maximum effort. Thus, it should cover a large range (1 to 2,000 Gm.) within a small margin of error.
2. The mouthpiece should be of small dimensions, so as to avoid displacement and stretching of the tissues.
3. The recordings should not be influenced by proximity of metallic appliances, fluctuations of line voltage or temperature, and moisture changes.
4. The mechanism should be simple to operate, and permit routine application without discomfort to the patient.

PRINCIPLE OF THE INSTRUMENT

With these objectives in view, we have developed a new instrument for recording oral muscle forces and have termed it the *photoelectric myodynagraph*. The instrument was evolved after our experience had demonstrated two other specially designed instruments to be inadequate in our hands. One was based on the principle of hydrostatics, and the other was a modified strain gauge.

The photoelectric myodynagraph has an enclosed column of gas (air) at atmospheric pressure connected to a photoelectric recording mechanism. Pressure on the compressible mouthpiece located at one end raises the pressure

This instrument was developed for a study on oral muscle forces supported, in part, by a Research Grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

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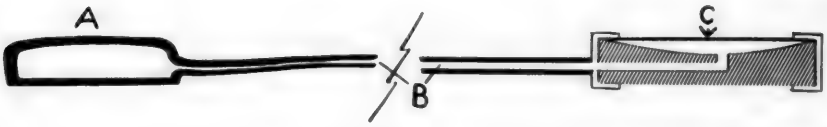


Fig. 1.—Diagram illustrating the principle of the myodynagraph. *A*, Compressible rubber mouthpiece; *B*, enclosed column of air at atmospheric pressure; *C*, rubber diaphragm.

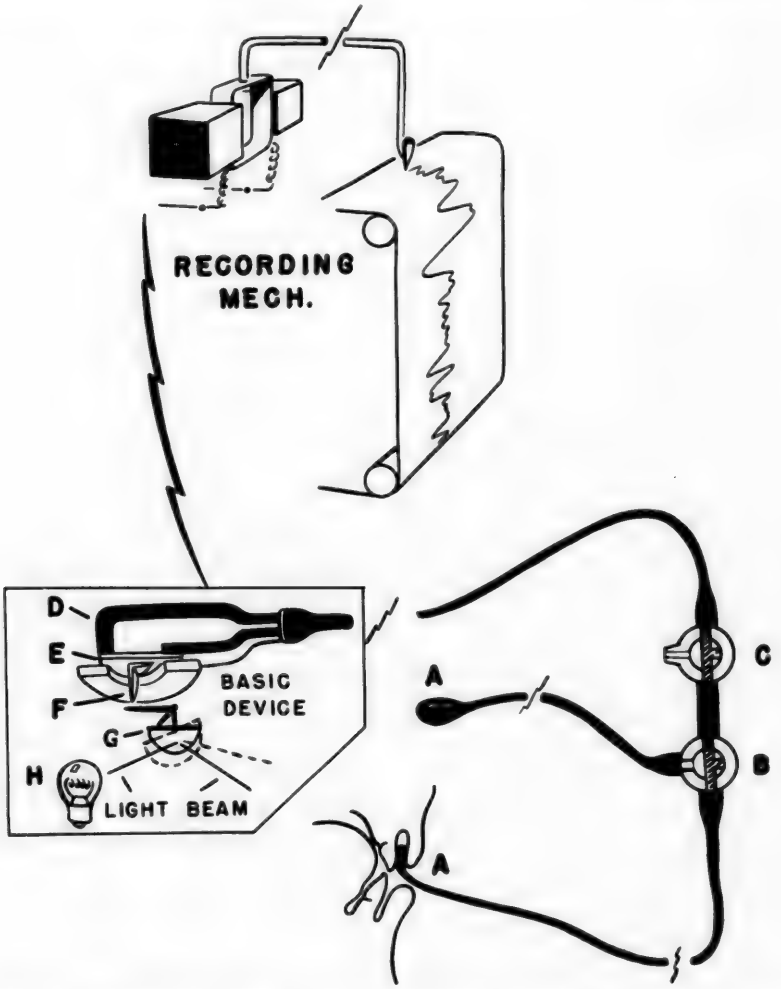


Fig. 2.—Diagrammatic sketch of the photoelectric recorder. *A*, Mouthpiece; *B*, first three-way stopcock; *C*, second three-way stopcock; *D*, metal chamber; *E*, rubber diaphragm; *F*, metal arm cemented on the rubber diaphragm; *G*, mirror of the basic device; *H*, light source.

of the air column. At the other end, a thin rubber diaphragm is located, which by distention indicates variations of pressure in the system (Fig. 1). This rubber diaphragm in turn deflects the mirror of the basic device of the photoelectric recording system, thus making a record of the magnitude of pressure applied at the mouthpiece (Fig. 2).

Fig. 3.—Photograph of the mouthpiece in side view (right) and front view (left).

DESCRIPTION OF THE COMPONENTS

The instrument, mounted on an eighteen by twenty-four inch table, consists essentially of five components. These are: (1) *two mouthpieces* connected by suitable leads to (2) *two three-way stopcocks,* which are connected to (3) *the basic device.* The basic device forms the link between the pressure system and the recording mechanism. Pressure applied on the mouthpiece is transmitted through the column of air to the rubber diaphragm, which deflects the mirror of the basic device. The basic device is enclosed within (4) *the photoelectric recorder,* which is activated by deflection of the mirror. Recordings are

made on a moving roll of paper. (5) *A conversion device* permits recordings to be converted in terms of force per unit area.

The Mouthpiece.—The mouthpiece consists essentially of a molded rubber bellows with a 0.040 inch stiff rubber tubing lead (Fig. 3). A special type of

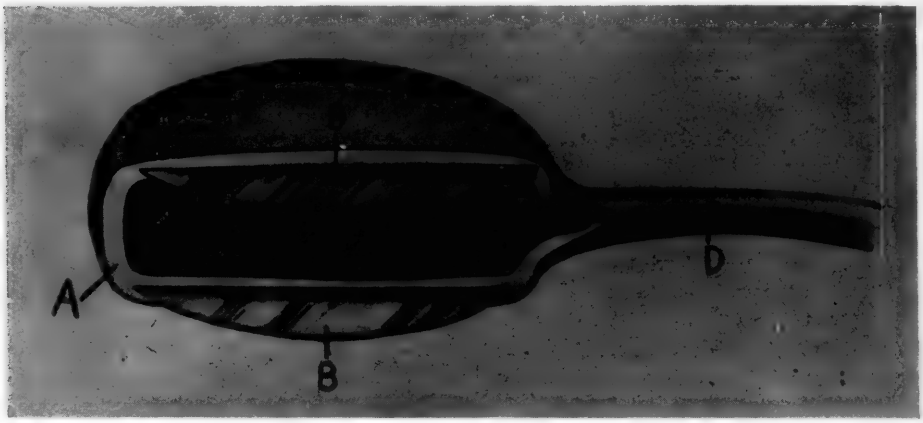


Fig. 4.—Diagrammatic section of the mouthpiece. *A*, Molded rubber walls; *B*, metal plates embedded in the walls; *C*, air cavity of the mouthpiece; *D*, stiff rubber tubing lead.

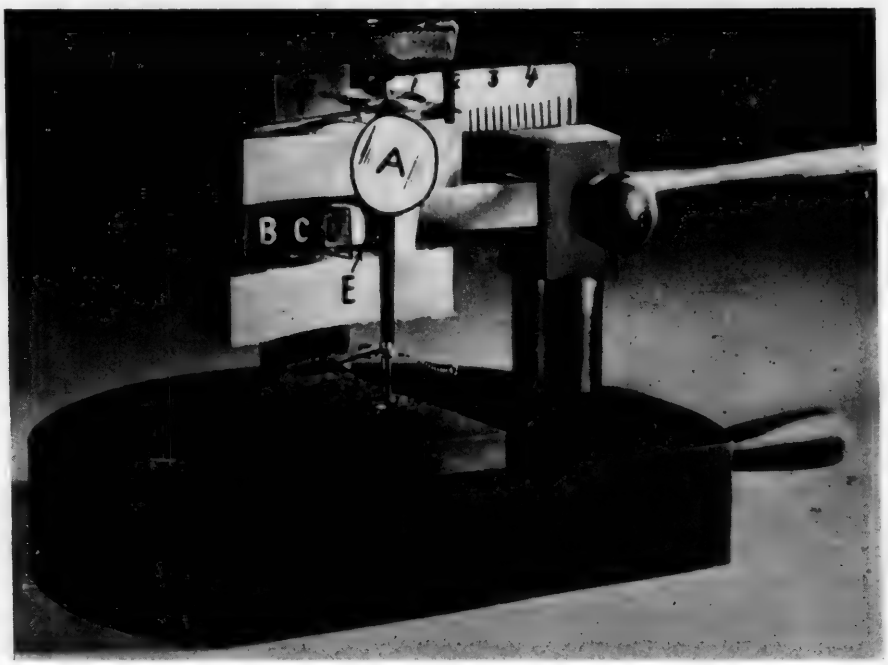


Fig. 5.—Photograph of the basic device. *A*, The mirror; *B*, metal chamber; *C*, rubber diaphragm; *D*, metal arm cemented on the rubber diaphragm; *E*, lateral extension of the mirror mount; *F*, watch spring of the mirror mount.

rubber (No. 63 Natural*) is used to make the mouthpiece. Two thin metal disks, 6 mm. in diameter, are embedded in each mouthpiece (Fig. 4). For the lower ranges, a thin-walled mouthpiece, made from a more resilient type of rubber, gives a high degree of sensitivity. For higher ranges, a relatively thick-walled mouthpiece, made from stiffer rubber, is used. The type of rubber used in the construction of mouthpieces suffers no measurable permanent deformation, and returns to its original shape after the pressure is removed.

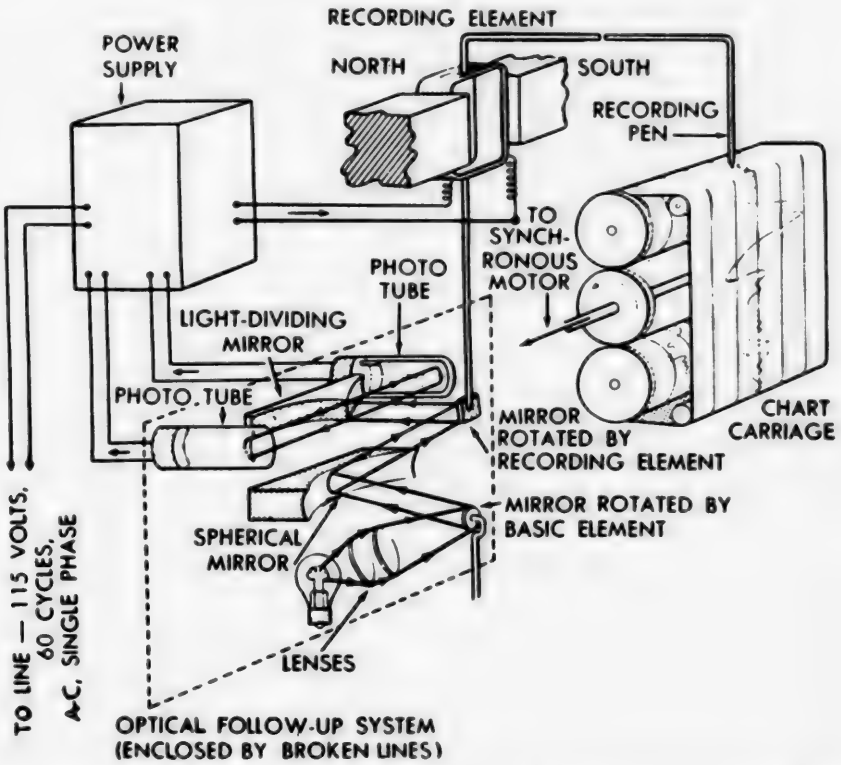


Fig. 6.—Diagrammatic representation of the mechanics of the photoelectric recorder. (From General Electric Company, Cat. No. GEC-2541.)

The mouthpiece can be modified for use in measuring other forces (for example, biting forces). In our study on muscle forces of tongue and lips, two types of mouthpieces are used: 1. A low-range mouthpiece for the lips. Its external dimensions are 8 mm. in diameter and 3.5 mm. in maximum thickness. Its sensitivity range is 2 to 750 Gm., with an average 6 per cent error. 2. A high-range mouthpiece for the tongue. Its external dimensions are 10 mm. in diameter and 4 mm. in maximum thickness. It records forces from 5 to 2,000 Gm., with an average 5 per cent error (calibration errors).

*Major Chemical Works, Boston, Mass.

The mouthpieces are provided with nonsensitive metal backings and suitable handling devices. The leads from the mouthpieces are connected to polyethylene tubes (PE 240*), which resist compression and tolerate usual handling without distortion.

Fig. 7.—Photograph of the conversion device. *A*, Base; *B*, the mouthpiece; *C*, hinge lever; *D*, metal disk resting on the mouthpiece; *E*, weights suspended on the end of the hinge lever.

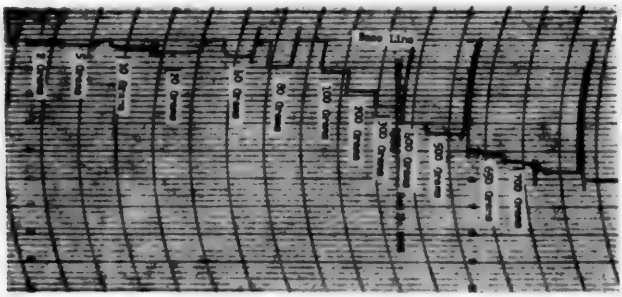
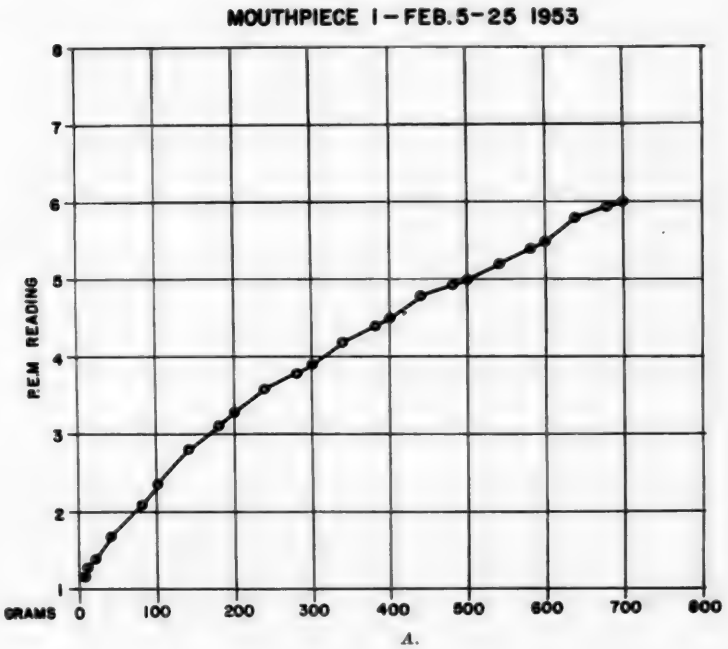
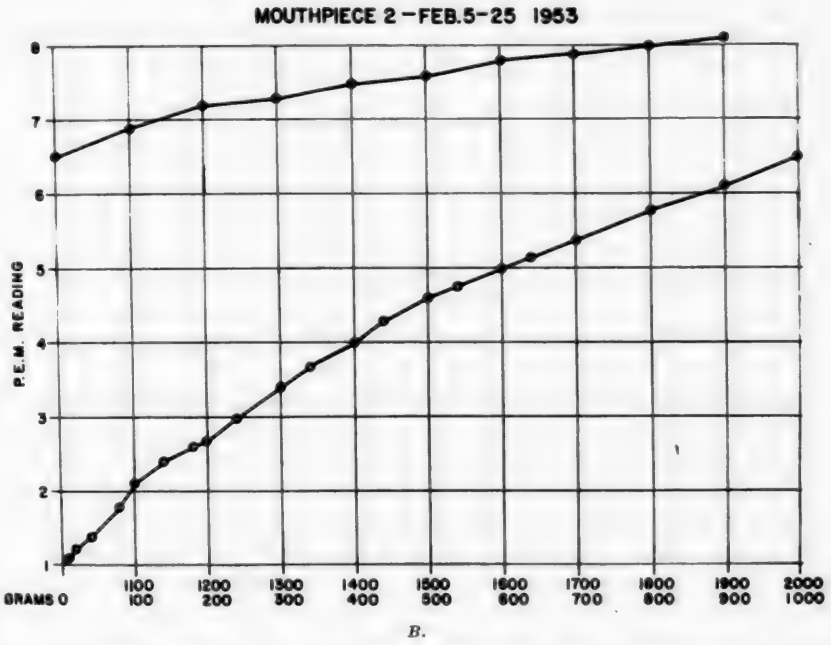


Fig. 8.—Calibration; recordings made with known weights.

*Clay Adams Company, Inc., New York, N. Y.



A.



B.

Fig. 9.—Calibration graph of (*A*) the low-range mouthpiece used for measurement of lip forces and (*B*) the high-range mouthpiece used for measurement of tongue forces.

The Three-way Stopcocks.—The two mouthpieces are connected via two three-way stopcocks to the tambour of the basic device. This arrangement permits the operator to connect either of the mouthpieces to the photoelectric recorder or to the external atmosphere by simple manipulation of the stopcocks

The Basic Device (Fig. 5).—The basic device includes a mirror (*A*) and a small metal chamber (*B*) with a thin rubber diaphragm (*C*) clamped on air-tight. A small metal arm (*D*) cemented on the rubber diaphragm rests against the lateral extension of the mirror mount (*E*). Distention of the rubber diaphragm causes deflection of the mirror. The sensitivity and range of the instrument can be controlled by altering the distance at which the metal arm (*D*) rests against the lateral extension of the mirror mount (*E*). A watch spring (*F*) on the mirror mount causes the mirror to return to its original position when the rubber diaphragm is deflated on removal of pressure on the mouthpiece.

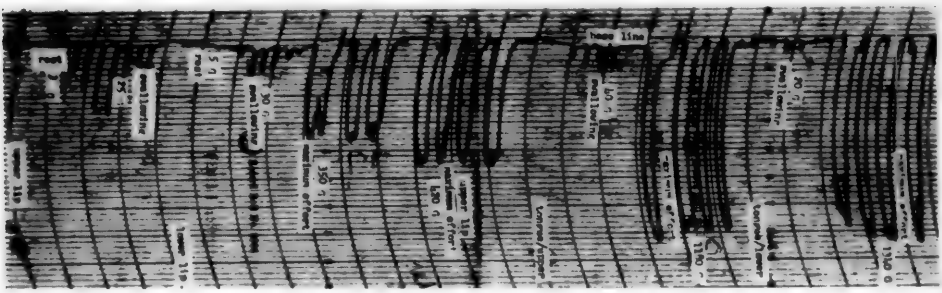


Fig. 10.—Sample recording of lip and tongue forces.

The enclosed column of air in the system is at atmospheric pressure. Force applied at the mouthpiece is transmitted through the air column to the thin rubber diaphragm of the basic device, causing distention of the diaphragm. This deflects the mirror of the basic instrument, causing a response of the photoelectric recording mechanism.

*The Photoelectric Recorder.**—The shaft of the recording mechanism also carries a small mirror, which is on the same vertical line as the mirror of the basic device (Fig. 6). When the horizontal axes of these mirrors are parallel, the light (from the incandescent lamps of the photoelectric recorder) is equally divided between two phototubes; hence, there is no flow of current through the recording coil. When the mirror in the basic device is deflected by distention of the rubber diaphragm, there is a shift of light from one phototube to the other. This results in a flow of current through the recording coil, which in turn moves the recording pen to a position where the two mirrors are again parallel.

The recording mechanism is of permanent-magnet moving-coil design, having high torque and rapid response. The recording pen responds rapidly and precisely to the deflection of the mirror of the basic device. The recordings are

*General Electric. Type CE.

made on a record roll with ten major and fifty minor divisions. The chart carriage is equipped with direct gearing, assuring positive and accurate timing. The chart speed is adjustable.

The Conversion Device (Fig. 7).—The conversion device is a simple arrangement of a base (*A*) upon which the mouthpiece (*B*) is placed. A hinge lever (*C*) extends over the mouthpiece. From this lever a metal disc, 10 mm. in diameter (*D*), rests over the mouthpiece. Weights (*E*) are suspended on the free end of the lever. The ratio of the work arm to the power arm is 1:2. Thus, the weight applied at the end and the resulting pressure on the mouthpiece are in the same proportion. Each mouthpiece is calibrated (Fig. 8), and a graph made from the mean of three calibrations (Fig. 9). Subsequently, the patient's recordings are transcribed in terms of grams by reference to these graphs (Fig. 10).

Procedure for Temperature Correction.—The proper mouthpiece is selected and connected to the external atmosphere by means of the three-way stopcocks. It is then placed in the patient's mouth for ten to fifteen seconds to permit expanded air (due to temperature rise) to be expelled to the outside atmosphere. Thus, the air column in the instrument is maintained at atmospheric pressure. The mouthpiece is then connected to the recorder by turning the second three-way stopcock a one-quarter turn.

OPERATION OF THE PHOTOELECTRIC MYODYNAGRAPH

1. The power switch to the photoelectric recorder is turned on, and the switch for chart feed is set at slow.
2. The three-way stopcocks are manipulated to connect the selected mouthpiece to the outside atmosphere.
3. The patient is seated with the Frankfort horizontal plane parallel to the floor, and is asked to relax. The mouthpiece is placed in the patient's mouth in the desired position.
4. A ten to fifteen seconds' waiting period is necessary for the expulsion of air which is expanded due to rise in temperature.
5. The chart feed is turned to fast. The patient's lip is lifted momentarily from the mouthpiece and the second stopcock turned a one-quarter turn, connecting the mouthpiece to the recorder. Recordings are then made.
6. After the recordings are completed, the mouthpiece is removed from the patient's mouth and connected to the external atmosphere by means of the second three-way stopcock. The feeding switch is turned to slow.
7. The mouthpiece is sterilized in 95 per cent alcohol, and the equipment is ready for the next recording.

The photoelectric myodynagraph has been in use in myometric studies for a period of eighteen months. Repeated calibrations with known weights have demonstrated the instrument to be accurate and precise, with an average 6 per cent error. It is simple to operate and permits routine application. The rubber elements last for several months without measurable change in performance. At present, we have obtained orthodontic and myometric records of 140 children,

with a view to correlating these data. The orthodontic records include cephalographic data obtained with Margolis' cephalostat and x-ray fixator.[11] This study is being continued to include a total of 200 children. Also, a study on the effect of myofunctional therapy on oral musculature is in progress. Another group of seventy-five children, receiving orthodontic treatment, is evaluated at eight-week intervals to determine the influence of orthodontic tooth movements on oral musculature. A report on the findings of these studies will be presented at a later date.

We wish to acknowledge the advice of E. Orowan, D. Eng., F.R.S., professor in the Department of Mechanical Engineering, Massachusetts Institute of Technology, in the development of this instrument. The technical assistance of Mr. Walter Culver, Department of Pharmacology, Tufts College Medical School, is also gratefully acknowledged.

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FREQUENCY, SIZE, AND BILATERALISM OF CARABELLI'S TUBERCLE

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THE permanent maxillary first molar sometimes has "an elevation of enamel" (Schwartz[1]) on "the lingual side of the mesiolingual cusp, about midway between its summit and the neck of the tooth" (Broomell[2]). This protuberance is referred to variously as a "fifth lobe" (Black[3]), a "supplemental cusp" (Wheeler[4]), an "accessory cusp" (Sicher[5]), a "mesiolingual elevation or prominence" (Diamond[6]), a "fifth cusp" (Broomell and Fischelis[7]), "Carabelli's tubercle" (Zeisz and Nuckolls[8]), and "Carabelli's cusp" (Stillson[9]).

PROBLEM AND ORIENTATION

Statement of Problem.—The present paper has a twofold purpose: (1) to review systematically the content in textbooks of dental anatomy pertaining to Carabelli's protuberance, and (2) to present original findings on the frequency, size, and bilateralism of this "elevation of enamel" in a sample of 100 children 9 years of age.

Elevation and Groove.—In its well-developed form, the supplemental lobe projecting from the lingual surface of the mesiolingual cusp is delineated occlusally "by a curved groove" (Sicher[5]). To quote Broomell and Fischelis,[7] "the groove begins near the center of the mesial surface and passes obliquely toward the summit of the mesiolingual cusp, before reaching which it makes an abrupt turn rootward, and joins the lingual terminal of the distolingual groove."

Wide variation is stated to exist in the extent of the groove and the size of the elevation. The former is described by Stillson[10] and Sicher[5] as ranging from a very highly developed groove to a mere pit representing the remnant of the groove. Variation in the latter, according to Zeisz and Nuckolls[8] and Diamond,[6] extends from being the largest cusp of the tooth to constituting a barely noticeable elevation.

FREQUENCY AND SIZE

Literature.—Textbook statements on the frequency with which an accessory mesiolingual elevation is found run from ambiguous notations to precise statements. Stillson[9] treated the topic vaguely, stating only that the permanent maxillary first molar may have a fifth lobe. Several others are only slightly more specific regarding frequency of occurrence; Black[3] commented that a fifth lobe is found in many examples, Schwartz[1] that it occurs in a large

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number of cases, Broomell[2] that it is frequently developed, Dewey[11] that it occurs in the majority, and Zeisz and Nuckolls[8] that it is often found but may be entirely absent.

Diamond[6] stated: "Statistically, about 50 per cent of the permanent [maxillary] first molar teeth of modern mixed Europeans are free of the mesiolingual elevation or tubercle." He explained that where the tubercle is not entirely absent, its degree of prominence varies from a barely noticeable elevation to a very marked prominence.

Some writers discussed frequency in terms of a scale extending from a highly developed fifth lobe, through a barely noticeable elevation, to a trace of the mesiolingual groove. Sicher[5] wrote: "A well-developed Carabelli's tubercle is found in from 10 to 15 per cent of all upper first molars" and a "small pit . . . the last evidence of this accessory element . . . in about 40 per cent of all upper first molars." Wheeler[4] expressed the view that some evidence of the mesiolingual groove is distinguishable in 100 per cent of permanent maxillary first molars.

Pedersen[12] and Shapiro[13] investigated the incidence of Carabelli's cusp among Mongoloids and Negroids, and Dietz[14] associated it with forms of the maxillary permanent first incisor.

Original Findings.—Preparatory to securing original findings for frequency and size, careful study was made of a series of fifty maxillary casts in which the permanent first molars were present. A series of notations was made on the different conditions observed, that is, absence of accessory features, evidence of minor furrows or pits, gradation in the size and delineation of lobar protuberances.

These notations provided the foundation for an initial attempt to derive a descriptive scale. Six categories were defined and their usability tested. Each of us re-examined the fifty maxillary casts used for preliminary work and rated the permanent right first molar in one of the six categories. From this preliminary study a revised scale was constructed consisting of four categories. The scale is described as follows:

Category 1: There is a moderate to large lingual elevation separated from the mesiolingual cusp by a well-demarcated, archlike groove. Viewed from either terminus, the groove passes obliquely toward the summit of the mesiolingual cusp, turns at a level below the summit, and then passes obliquely rootward. Included in this category are cases in which the groove is well marked except for a short interruption (a narrow confluence of accessory protuberance and mesiolingual cusp) at its crest.

Category 2: There is a moderate to large lingual elevation separated from the mesiolingual cusp by a well-demarcated, partial groove. The groove may be partial in that it extends buccally to (a) the mesial side of the elevation only, (b) the distal side of the elevation only, (c) one side of the elevation and slightly past its crest, or (d) part of both sides of the elevation.

Category 3: There is a slight elevation projecting from the lingual surface of the mesiolingual cusp. The elevation may vary in appearance from a small conical hillock to a low plateau outlined by a diminutive wall or groove. It may have a somewhat bifocated or two-lobed contour.

Category 4: There is no definite protuberance from the lingual surface of the mesiolingual cusp. The lingual surface may be smooth or its undulation of a character that does not lend itself to confident interpretation as an elevation. Among the irregularities included in this category are those which appear equally interpretable as shallow grooves in the surface or as low ridges on the surface.

This scale was applied to maxillary dental casts taken on a sample of 100 white children 9 years of age. The children, fifty boys and fifty girls, were of northwest European descent and resided in or near Iowa City, Iowa. They had participated for several years in a long-term study of facial growth. Enrollment in the study was based on willingness to cooperate and probability of continuing residence in the community; it was not related to the dental needs of the subjects.

Age 9 years was chosen because, on the one hand, this was the earliest age at which the permanent maxillary first molars were erupted in 100 per cent of the subjects, and, on the other hand, it was a sufficiently young age to minimize the likelihood of enamel features having been "erased by frictional wear."[4]

The rating procedure was as follows: (1) the 100 permanent maxillary right first molars were classified first by E. H. H. and then, independently, by H. V. M.; (2) the 100 left first molars were classified by both raters working independently; and (3) instances of rating disagreement were subjected to classification a second time by each investigator.

For the 200 teeth taken together, the initial category placements of both of us coincided 146 times, that is, there was 73 per cent agreement. The disagreements were all between adjacent categories, except for a single discrepancy of two categories.

TABLE I

RATINGS OF THE LINGUAL SURFACE OF THE MESIOLINGUAL CUSP OF THE PERMANENT FIRST MOLAR WITH REFERENCE TO THE OCCURRENCE, SIZE, AND DEMARCATION OF AN ACCESSORY PROTUBERANCE (SEE TEXT FOR DESCRIPTION OF THE SAMPLE AND DEFINITION OF THE CATEGORIES)

GROUP	NUMBER OF SUBJECTS	NUMBER OF TEETH	CATEGORY FREQUENCIES			
			1	2	3	4
Total series	100	200	74 (37%)	45 (22.5%)	48 (24%)	33 (16.5%)
Right molars	100	100	37	23	23	17
Left molars	100	100	37	22	25	16
Boys	50	100	40	26	24	10
Girls	50	100	34	19	24	23

The frequencies obtained for each category are displayed in Table I. Almost 60 per cent of the mesiolingual cusps studied had a lingual projection of moderate size or larger, approximately 84 per cent exhibited some amount of supplemental protuberance, and about 16 per cent presented no unequivocal indication of Carabelli's prominence. For the right and left sides of the maxillary dental arch, the frequencies in each of the four categories are not significantly different. An accessory elevation on the lingual surface of the

mesiolingual cusp is found for 83 per cent of the 100 right molars examined, and for 84 per cent of the 100 left molars. There is a slight tendency for boys to exhibit more large to moderate protuberances than girls, and for girls to exhibit more instances of lack of an enamel elevation than boys. This trend is not statistically significant; a chi square of 6.70 was obtained, whereas a value of 7.82 is required before the inference of a sex difference becomes tenable at the 5 per cent confidence level.

The largest accessory elevation found among the fifty boys and the largest among the fifty girls were subjected to careful measurement. These data are of interest (1) in connection with the foregoing discussion of sex differences and (2) in relation to the statement by Zeisz and Nuckolls[8] that the cusp of Carabelli may be the largest cusp of the tooth. The largest cusp for a boy measured 5.5 mm. in maximum mesiodistal diameter, projected 3.0 mm. lingually from the surface of the mesiolingual cusp, and lacked approximately 1.5 mm. of extending occlusally to the level of the mesiolingual cusp. Corresponding measurements on the largest protuberance for a girl are 4.5 mm., 2.0 mm., and 2.0 mm., respectively.

BILATERALISM

Literature.—Some texts state that, when present, Carabelli's tubercle is invariably bilateral[3, 6, 9]; others describe it as usually bilateral.[2, 8]

Research Findings.—Bilateralism was investigated on the 100 9-year-old subjects in two ways: (1) by bivariant analysis of the previously described ratings and (2) through direct ratings on bilateralism from simultaneous study of the right and left sides of each cast. In the first approach, the independent right and left ratings of the permanent maxillary first molars were distributed in a double-entry table. The columns of the table represented the right side of the dental arch, and the rows the left side. In the event a child was rated 1 for the right molar and 2 for the left molar, he was placed in the cell below the column designated 1 and to the right of the row designated 2. The results obtained for the full series of 100 children are presented in Table II. Roman numerals have been used in entering four of the cells (those that would include every individual under the hypothesis of invariable bilateralism) and Arabic numerals in entering other cells (those that portray departure from bilateralism).

TABLE II

RELATION OF THE RIGHT AND LEFT SIDES OF THE MAXILLARY DENTAL ARCH WITH REFERENCE TO OCCURRENCE, SIZE, AND DEMARCATION OF CARABELLI'S TUBERCLE (COLUMN FREQUENCIES REPRESENT THE LEFT SIDE AND ROW FREQUENCIES THE RIGHT SIDE) (SEE TEXT FOR SPECIFICATION OF THE RATING CATEGORIES)

		RIGHT			
	CATEGORY	1	2	3	4
LEFT	1	XXVIII	7	2	
	2	7	VIII	7	
	3	2	6	X	7
	4		2	4	X

Proceeding by columns, Table II shows: (1) Of the 37 children whose permanent maxillary right first molar was placed in Category 1, the left molar ratings were 1 for 28 children, 2 for 7 children, and 3 for 2 children. (2) Of the 23 children whose right molar was asigned to Category 2, placement of the left molar ranged from Category 1 in seven instances to Category 4 in two instances. (3) Of the 23 children whose right molar was rated as representing Category 3, only 10 were assigned to Category 3 by an independent rating for the left molar. (4) Of the 17 children whose right molar was considered to lack irrefragable evidence of an accessory elevation, 7 were observed to have a small supplementary elevation on the left molar.

It is obvious that findings also can be drawn from Table II by rows, and diagonally. Findings by rows are of the same order as those by columns, the essential difference being a shift to the discussion of right molar variation within left molar subgroups. Findings from diagonal examination are: (1) Only 56 of the 100 children (less than 60 per cent) fall in the 4 cells required under the hypothesis of invariable bilateralism. (2) Thirty-eight of the children (almost 40 per cent) differ from expectation under the hypothesis of invariable bilateralism by one cell or category. (3) Six per cent of the children deviate from expectation under the hypothesis of invariable bilateralism by as much as two categories.

Direct ratings for bilateralism were made by reobserving each dental cast and simultaneously appraising the right and left lingual surfaces of the permanent first molars. Appraisal consisted in determining whether the accessory elevation was larger on the right mesiolingual cusp, larger on the left mesiolingual cusp, or not macroscopically different for the two cusps. As before, we worked separately, both rating the entire series of maxillary casts. Tabulation was made of agreements regarding the existence and direction of a difference between the right and left sides. The right elevation was larger than the left in 40 subjects (22 boys and 18 girls), and the left elevation larger than the right in 30 subjects (14 boys and 16 girls). Generalizing, there was a noticeable (and confirmable) quantitative difference between the elevation on the two sides in 70 per cent of the 100 subjects.

SUMMARY

A study was made of the occurrence, size, and bilateralism of Carabelli's tubercle in 100 physically normal, 9-year-old Iowa children of northwest European ancestry. The basic data were controlled observations on dental casts, that is, observations of the lingual surfaces of the mesiolingual cusps of the permanent maxillary first molars.

Eighty-four per cent of the 200 first molars examined were found to show some amount of supplemental protuberance. Approximately 60 per cent had moderate to large prominences, while 16 per cent lacked any observable accessory elevation. The largest tubercle in the series measured 5.5 mm. in maximal mesiodistal diameter, projected 3.0 mm. lingually from the surface of the mesiolingual cusp, and lacked approximately 1.5 mm. of extending occlusally to the level of the mesiolingual cusp.

Thirteen per cent of the children exhibited no accessory elevation on one side of the arch and a slight to moderate elevation on the other. There was an observable difference in the size of the tubercle on the right and left sides for 70 per cent of the subjects.

Acknowledgment is made to Dr. L. Bodine Higley for reading the manuscript and taking all of the dental impressions.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH 1955 MEETING

The 33rd General Meeting will be held at the Morrison Hotel, Chicago, Illinois, March 18, 19, and 20, 1955, in conjunction with the annual meeting of the American Association of Dental Schools, March 21-23.

The closing date for acceptance of items for the program will be Dec. 1, 1954.

NOVICE AWARD

The Novice Award, which carries a prize of $100, will be offered again at the 1955 meeting. Details will be published in the August JOURNAL.